Cranfield University at Silsoe

Institute of BioScience & Technology (IBST)

Postharvest Technology Group

Ph.D. Thesis

Academic Year 2002 – 2003

Lord Abbey

Discrimination of *Allium* Headspace Volatiles Affected by Variations in Genotype, Growing Environment and Storage Using an Electronic Nose

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The thesis is submitted in fulfilment of the requirement of the Degree of Doctor of Philosophy

ABSTRACT

Alliums are valued mainly for their unique organosulphur-derived flavours and aromas. Traditional sensory and analytical determinations of *Allium* quality are constrained by high cost, technical difficulties and, time and human limitations. This thesis investigates the potential for use of relatively novel electronic nose (E-nose) technology for *Allium* discrimination. Chapters 3, 4 (Sections 4.1 to 4.3), 5 (Sections 5.1 and 5.2) and *Appendices* II and III inclusive have been published or submitted for publication. Consequently, Chapters in this thesis are presented in the form of papers.

The E-nose AromaScan LabStation A32/8S (Osmetech Plc., UK) consists of 32 conducting polymer miniature sensors. Adsorbed odour molecules alter the electric conduction mechanism of the sensor polymer. The response is measured as proportional (%) change in sensor resistance ratio (%dR/R).

The E-nose discriminated *Allium* types (Chapter 3), varieties of spring onion grown with or without sulphur addition and a single variety of spring onion grown under different levels of sulphur, nitrogen, water-deficit stress and soil type (Chapter 4). Bulb onion affected by nitrogen, sulphur and soil type and diced onion sealed in polyethylene bags stored at 4°C for 9 days were also discriminated by the E-nose (Chapter 5). A descriptive model for the direction of E-nose sensor polymer response to *Allium* headspace volatiles affected by genotypic differences and edaphic variables was outlined in Section 6.2.

Principal Component Analyses (PCA) of E-nose data sets output accounted for >75% to nearly 100% of variations in the Alliums. The variations in *Allium* genotype differentially affected the E-nose sensor conductivity following headspace volatiles interaction with sensor polymer element. Classification of data sets output showed greater (Mahalanobis distance statistic, $D^2>3.0$) sensitivity of spring onion cvs Guardsman and Fragrance to S fertilisation while the headspace volatiles characteristics of cvs Winter Over and Paris Silverskin were not significantly ($D^2<3.0$) altered by S. The headspace volatiles of onion bulb cv. Sprinters also responded to S fertilisation ($D^2>3.0$) and thus, increased %dR/R. Overall, N-fertilised onion cv. Sprinters reduced E-nose sensor conductivity leading to an increase in

%dR/R. Increases in water-deficit stress i.e. ≥ -0.80 MPa soil water potential, SWP generally reduced separation between E-nose data set clusters for clay versus sandy loam soils from $D^2 = 43.2$ for -0.01 MPa SWP to $D^2 = 6.2$ for -1.19 MPa. Headspace volatiles of onions grown in the glasshouse clay increased %dR/R compared to reduced %dR/R values for both glasshouse and field sandy loam soils. The E-nose detected gradual changes in headspace volatiles of diced onion wrapped in polyethylene bags stored at 4°C for 9 days. The changes in headspace volatiles reduced %dR/R values while data set cluster separations with reference to day 0 for each sampling time increased from $D^2 = 3.6$, 5.8 and 7.0 on days 3, 6 and 9, respectively.

The results suggested that *Allium* quality can be assessed with ease along production, postharvest and marketing chains compared to traditional destructive methods. Linear correlations for E-nose data sets versus *Allium* pungency determinants (pyruvic acid and lachrymatory potency), total soluble solids and dry-matter were poor. The thesis discusses the commercial significance of the result and its implication for the development of E-nose sensor tailored for Alliums. This would promote application and use of E-nose technology in the *Allium* industry, germplasm evaluation, and discrimination of agronomic variables and possibly, monitoring spoilage pathogens during storage.

The effects of nitrogen, sulphur, water-deficit stress and soil type and their interactions have given new insight into agronomic inputs on growth and microbial load (Chapters 4.3, 5.1 and *Appendix* III).

DEDICATION

I dedicate this Ph.D. thesis to my dear wife Mrs Mercy Abbey and children, Precious Daphne Anettey Abbey Jr., Darren Shamo Abbey and Deva Naa Abeley Abbey.

ACKNOWLEDGEMENTS

The author acknowledges Prof. Daryl C. Joyce for his invaluable contribution and guidance from the initial proposition of this study up to its implementation. Similarly, Dr. Julia Aked is acknowledged for her input. Special thanks go to Dr. Lesley Currah who introduced the author to Cranfield University and Dr. Julia Aked. The author is grateful for the interest shown and the timely assistance rendered by Dr. Leon A. Terry who supervised the write-up of the thesis.

The author wish to express his gratitude to the Association of Commonwealth Universities and the Ghana Scholarship Secretariat for awarding the Ph.D. scholarship and also, to Crops Research Institute (Council for Scientific and Industrial Research), Ghana for granting the study leave.

Parripak Foods Ltd. (UK) is acknowledged for supplying *Allium* samples. The author appreciates the advice offered by Messrs Andrew Tummon (Osmetech Plc., Crewe, UK) on the electronic nose, Brian Smith (HRI) on agronomic work, Charles Marshall (NSSR) for advice on statistics, Allen Hilton (Postharvest Department) for technical support and Bob Walker (Farm manager) for field assistance. Many thanks go to Dr. Robin Matthews and Prof. Naresh Magan who acted as Thesis Committee Chairmen at different stages of the study for their criticism and suggestions. Special thanks go to Mrs Linda Chapman who assisted in printing of the thesis. The others who require mention are Drs. Agnes Simpson Budu, Malkanthi Daundesikera and Mr. Paul Dauny for various forms of assistance and encouragement.

The author appreciates the moral and spiritual support given by all family members, especially his dad Mr. Precious A. Abbey Snr., Mrs. Juliana Abbey, Ms. Sarah B. Wang, siblings, Mr. C. K. Mingle and Ms. Sarah M. Acquaye.

"I AM" so grateful to my "Beloved Mighty I AM Presence" for all we have received and all that is yet to come! GOD BLESS YOU!!

TABLE OF CONTENTS

LISTS OF TABLES

Chapter 2

Chapter 3

Chapter 4 (Section 1)

Section 2

Section 3

Chapter 5 (Section 1)

Section 2

LISTS OF FIGURES

Chapter 2

Chapter 3

Chapter 4 (Section 1)

Figure 1. Leaf greenness (A) and plant height (B) of spring onion cultivar White Lisbon grown on clay (filled circles) and sandy loam (open circles) and supplied with different amounts of sulphur (S). The vertical bars represent standard errors of the means (n = 4)

Figure 2. Fresh weight and dry-matter production of spring onion cultivars 'White Lisbon' grown on clay (A, C) and sandy loam (B, D) at applied sulphur levels of 0.0 (filled circles), 2.9 (open circle) and 5.8 (filled triangle) kg ha^{-1}. The vertical bars represent standard errors of the means (n = 4)

Section 2

Figure 1. A 2D PCA plot for E-nose data clusters for headspace volatiles of eight spring onion genotypes grown on sandy loam at S nutrition levels; 0.0 (A) and 5.8 (B) kg S ha^{-1}. Cultivars: WO, Winter Over; PS, Paris Silverskin; WL, White Lisbon; SB, Sydney Bunching; G, 'Guardsman' (hybrid); F, Fragrant; WW, Winter White; EB, Egyptian Bunching (n = 5)

Figure 2. A 2D PCA plot for E-nose data clusters for headspace volatiles of spring onion cultivar White Lisbon grown on clay (C) and sandy loam (SL) at S nutrition levels of 0.0, 2.9 and 5.8 kg ha^{-1} (n = 4)

Section 3

Figure 1. Relative water content (A, B) and leaf water potential (C, D) of spring onion cv. White Lisbon grown on clay (filled circle) and on sandy loam (open circle) at different soil water potential levels before (A, C) and after (B, D) 24 hr of irrigation. The vertical bars represent standard error of the means (n = 16)

Figure 2. Green leaf length (A) and plant height (B) of spring onion cv. White Lisbon grown on clay (filled circle) and on sandy loam (open circle) at different soil water potential levels. The vertical bars represent standard error of the means (n = 16)

Figure 3. Fresh weight (A, B) and dry matter production (C, D) of spring onion cv. White Lisbon grown on clay (A, C) and on sandy loam (B, D) at soil water potential

LISTS OF ABBREVIATIONS AND SYMBOLS

*	significant at P<0.05
**	significant at P<0.01
%	per cent
dR/R	change in E-nose sensor resistance ratio
^{o}C	degree celsius
^{o} N	degree north
^{o} S	degree south
>	greater than
<	less than
≥	greater than or equal to
≤	less than or equal to
=	equal to
≠	not equal to
±	plus or minus
μ	micro (10^{-6})
ACC	1-aminocyclopropane-1-carboxylate
ACSO	S-alk(en)yl-L-cysteine sulphoxide
Al	aluminium
ANN	Artificial Neural Network
ANOVA	Analysis of Variance
B	Boron
BAW	Bulk Acoustic Wave
C_3H_6S	1-propenyl sulphenic
C_2H_3OCOOH	pyruvic acid (μmole g^{-1} fresh weight)
C_4H_5S	thiophene
C_4H_5N	pyrrole
C_6H_7N	aniline
CPGTH	carboxypropyl glutathione
CA	Control Atmosphere
ca	approximately
CEC	Cation Exchange Capacity (cmole kg^{-1})
CFU	colony forming unit (ml^{-1})

cm	centimetre
cmole	centimole
CO_2	carbondioxide
cv(s)	cultivar(s)
D^2	Mahalanobis distance statistic
Da	Dalton
DNPH	dinitrophenylhydrazine
DS	decisiemen
EC	electrolyte conductivity (dS m^{-1})
E-nose	electronic nose
Fe	iron
FMOC	fluoroenylmethyl chloroformate
g	gramme
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
h	hour
H_3PO_4	metaphosphoric acid
H_2O	water
ha	hactre
HPLC	high-performance liquid chromatography
J	joule
K	potassium
KCl	potassium chloride
kPa	kiloPascal
l	litre
LF	lachrymatory factor (thiopropanal sulphoxide)
LSD	Least Significant Difference
LWP	leaf water potential
m	metre
M	Molar
MA	modified atmosphere
meq	milliequivalent
MCSO	S-methyl-L-cysteine sulphoxide
mg	milligramme

MgO	magnesium oxide
min	minute
ml	millilitre
mm	millimetre
MOSFET	metal oxide semiconductors field effect transistors
MPa	megaPascal
MT	metric tonne
N	nitrogen
NaOH	sodium hydroxide
NH_3	ammonia
NH_4	ammonium
nl	nanolitre
nm	nanometre
NO_3	nitrate
NS	not significant at P>0.05
O_2	oxygen
P	phosphorus
PARC	pattern recognition
PCA	Principal Component Analysis
PCSO	S-propyl-L-cysteine sulphoxide
PRENCSO	S-(1-propenyl)-L-cysteine sulphoxide
QCM	quartz crystal microbalances
r	coefficient of correlation
R^2; r^2	coefficient of determination
RH	relative humidity
RWC	relative water content
S	sulphur
s	second
Si	silicon
Sn	tin
SO_4^{2-}	sulphate
SWP	soil water potential
TCA	trichloroacetic acid
TLC	thin layer chromatography

TSS	total soluble solids
v/v	volume by volume
WUE	water-use efficiency
w/v	weight by volume
w/w	weight by weight
Znose	electronic tongue
Zn	zinc

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

The genus *Allium* belongs to the family *Alliaceae*, which includes >500 species (Hanelt, 1990). Bulb onion (*Allium cepa* L.) is the most commercially important species among the Alliums (Brewster, 1994). World production of bulb onions rose from 3,625,254 MT on 212, 825 ha in 1997 to 4,184,835 MT on 224,851 ha in 2002 (FAOSTAT, 2002), thereby suggesting a worldwide rise in consumption. Other edible crop species of considerable commercial value include garlic (*A. sativum* L.), spring onion (*A. cepa* L.), shallot (*A. ascalonicum* auct. non Strand), leek (*A. ampeloprasum* L.) and chive (*A. tuberosum* L.).

Alliums are valued for their distinctive flavours (Block, 1992), which are comprised of basic taste indices such as sweetness and bitterness, mouthfeel and aroma (Meilgaard *et al.*, 1991) when eaten alone or added to other foods such as salads (Block, 1992; Randle, 1997a;). Alliums are also important for their medicinal properties (Block, 1992; Trueman *et al.*, 2001). Allium flavour is derived from four non-protein sulphur (S)-amino acid flavour precursor compounds, collectively termed S-alk(en)yl-L-cysteine sulphoxides (ACSOs; Lancaster and Kelly, 1983; Block, 1992). These ACSOs include S-methyl-, S-propyl-, S-1-propenyl- and S-2-propenyl-L-cysteine sulphoxides. Onion flavour as determined by pyruvate concentration (Schwimmer and Weston, 1961; Thomas and Parkin, 1994) increased with an increase in taste panellists score for pungency (DEFRA, 2001), but correlated poorly with individual sugars such as sucrose, glucose, fructose, total reducing sugars and fructans (Randle and Bussard, 1993a). Red- and purple-pigmented onions are rich in selenium, antioxidants and flavonoids, which have been reported to improve health and offer protection against cardiovascular diseases, cancer and atherosclerosis (Trueman *et al.*, 2001).

Fresh produce quality is determined prior to time of harvest due to many influential preharvest factors (Joyce and JianRong, 2002). The composition and amount of ACSOs vary among *Allium* genotypes (Freeman and Whenham, 1975a; Block *et al.*,

1992). Moreover, they can be modified by environmental variables and management practices (Randle, 1997a). Little is known about the interactive effects of these preharvest variables on *Allium* growth, quality and shelf life.

Traditional procedures for sensory and analytical tests of onion flavour are constrained by requirements of cost, time and/or expertise (Giese, 2000). Alternative fast and inexpensive methods would benefit the fresh produce and food industry, and also assist researchers in onion germplasm evaluation. The electronic nose (E-nose) is a fairly new device for sensing and discriminating among 'cocktails' of headspace volatiles. The E-nose has been successfully used to monitor the qualities of products in the food, perfume, brewery and pharmaceutical sectors (Kress-Rogers, 1997).

1.2 Aims

This study explores the potential use of an E-nose technology to discriminate *Allium* quality as affected by genotype, preharvest soil factors and postharvest storage duration. Associations between E-nose data sets versus both analytical and sensory tests for *Allium* flavour were also examined.

1.3 Thesis Structure

A literature search was conducted and determined that a considerable body of work on genotype, fertiliser, soil type, irrigation and climate effects on *Allium* growth and quality has been carried out (Chapter 2). Many of these reports were limited to bulb onion. Moreover, interactive effects of preharvest variables on postharvest flavour and non-flavour headspace volatiles were generally not reported. In this context, a review of E-nose technology for quality analysis of foods and food products and of the merits of E-nose over conventional flavour analysis methods is presented.

E-nose instruments fitted with different sensors have been used for assessing the quality of various food crops (Persaud and Talou, 1996; Bartlett *et al.*, 1997; Adechy *et al.*, 2000; Sinesio *et al.*, 2000), but not Alliums. Early unpublished work to differentiate onion bulb genotypes using E-nose was not successful (B. Smith, pers.

comm., 2000). This failure may possibly be attributed to the rapid changes in biochemical pathways that occur upon maceration of onions (Block, 1992). Sulphur and carbon compounds, which contribute to both flavour and non-flavour compounds of Alliums are also known to 'poison' or inhibit the interaction between vapour phase molecules and sensor elements (Kohl, 1997). In initial work, the author developed *Allium* sample preparation and handling procedures. More specifically, the optimum gas flow rate for sampling headspace gas and temperature and relative humidity settings for the E-nose sample station and sensor unit were determined (AromaScan service manual, 1998). The first experiment investigated the potential for use of a 32-conducting polymer sensor element E-nose (A32/8S AromaScan; Osmetech Plc., UK) for discrimination amongst five *Allium* types (Chapter 3). These *Allium* types were garlic, bulb and spring onions, shallot and leek.

Spring onions are popular in households, being eaten in fresh vegetable salad and for seasoning food. Comparisons of spring onion genotypes to variations in edaphic (soil) factors were evaluated in glasshouse experiments (Chapter 4). Interaction effects of eight spring onion genotypes by S fertilisation and one spring onion cultivar by soil type on growth and dry-matter yield were determined (Section 4.1). E-nose evaluation of these treatments effects and their interactions was investigated in follow up work to that on the different *Allium* types (Section 4.2). Part of the spring onion growing season in the UK is marked by high evapotranspiration and low rainfall, especially for all-year round cultivars (e.g. cv. White Lisbon; Anon., 1995). Fluctuations in soil moisture content generally reduce plant growth and yield as compared with regular watering (Momose and Kasubuchi, 2002; Hanson *et al.*, 2003). The degree of plant response to fluctuations in soil moisture content is likely to be dependent on soil nature. The effects of water-deficit stress and soil type on growth and dry-matter production of spring onion cv. White Lisbon, and the use of E-nose to discriminate treatment differences with reference to headspace volatiles were investigated (Section 4.3 and *Appendix* II).

Variations in soil properties can affect the association existing between nitrogen (N) and sulphur (S) assimilation reported generally for plants (Reuveny *et al.*, 1980). There is no evidence in the literature on the interaction effects of N, S and soil type on

onion bulb quality including flavour and headspace volatiles. The effects of these nutrients and soil type on the quality of onion bulb cv. Sprinters were determined using an E-nose and spectrophotometer determination of pyruvate concentration (Chapter 5). Responses of growth, bulb skin quality and other agronomic attributes were also investigated (*Appendix* III).

Minimal processing aims to preserve the freshness of food, including onions, at low cost and for long enough to allow distribution (Farkas, 2003). Unitisation, convenience, safety and quality of minimally processed onions drive increasing demand. Quality assurance is of paramount importance due to rapid deterioration of minimally processed fresh foods (King and Bolin, 1989; Farkas, 2003). Conventional sensory and analytical tests for onions, like for all other crops are not expedient (Payne, 1998). The E-nose may provide an alternative beneficial approach. The storage quality of diced onion wrapped in polyethylene bags was assessed over a period of 9 days using both E-nose and conventional quality control methods (Chapter 6).

CHAPTER 2: LITERATURE REVIEW

FACTORS INFLUENCING *ALLIUM* GROWTH AND QUALITY AND THE USE OF AN ELECTRONIC NOSE TO DISCRIMINATE ODOUR

2.1 Introduction

Onions (*Allium cepa* L.) are typically adapted to a wide range of varying agro-ecological zones that include both dry and humid sunny environments and also forest regions (Hanelt, 1990). Fig. 1 shows preharvest variables that affect crop production.

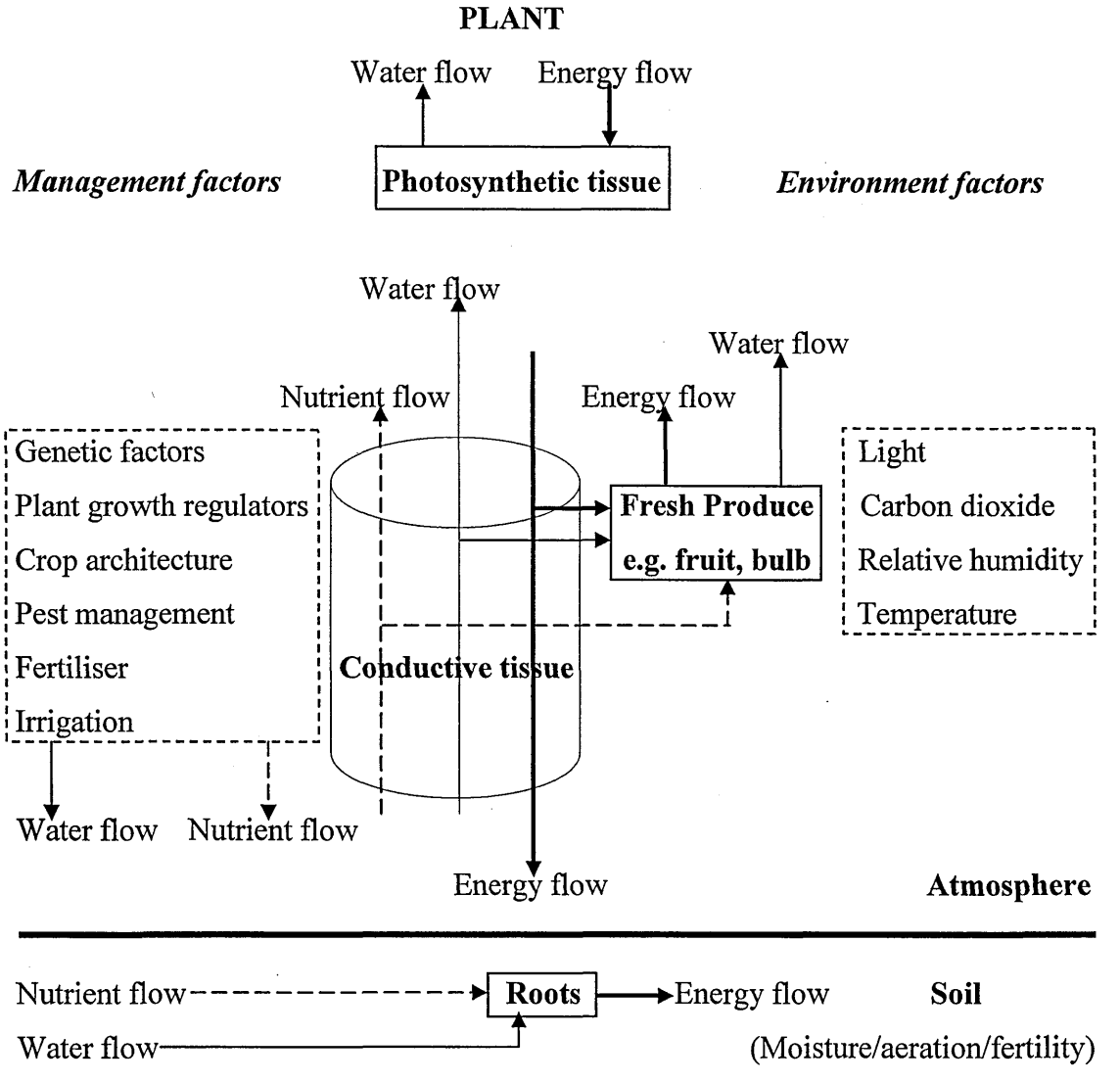

Figure 1. Preharvest variables that affect growth and quality of crops (redrawn from Beverly *et al.*, 1993).

Growth, harvest quality and storage ability of onions are, however, dependent on the genotype of individual cultivars and are modified by environment and management practices (Ashish *et al.*, 1995; Randle, 1997a; Debaene *et al.*, 1999; Boyhan *et al.*, 2001). Thus, variations in these variables can undesirably affect plant including onion growth, quality and storage ability, especially after severe preharvest and postharvest stresses (Fig. 1; Beverly *et al.*, 1993; Joyce *et al.*, 1998; Mattheis and Fellman, 1999).

Sensory test is certainly invaluable for fresh produce quality evaluation (Mattheis and Fellman, 1999). Onion quality is usually evaluated by human sensory appraisals, but is constrained by factors explained in Section 2.4.1. Electronic nose (E-nose) technology mimics the mammalian nose for smelling and has been used for monitoring qualities of both agricultural and non-agricultural products (Kress-Rogers, 1997). E-nose technology has proven promising and is, therefore, seen as a possible alternative to human sensory and/or analytical determinations of fresh produce quality.

2.2 Bulb Formation, Bulb Swelling, Bulb Dormancy and Nutrient Value

Bulbing in onion refers to changes in leaf morphology initiated when plants are exposed to critical daylengths at a minimum temperature (Rubatzky and Yamaguchi, 1997; see Section 2.2.2). Termination of leaf production marks the beginning of bulb formation (Fig. 2), during which period assimilates are translocated to the developing bulb (Brewster *et al.*, 1986; Iortsuun and Khan, 1989; Brewster, 1994; Ashish *et al.*, 1995).

The process of bulb swelling involves lateral expansion of cells in the lower third of the leaf sheaths (Brewster, 1994). During bulbing induction, sheath tissue concentrations of reducing sugars i.e. glucose and fructose increase, thereby decreasing the osmotic potential of the cells (Brewster, 1994). An osmotic gradient is created that draws water in to increase the size of the cells. Also, there is an increase in sink strengths of the leaf bases, which increases assimilate accumulation (Brewster *et al.*, 1986).

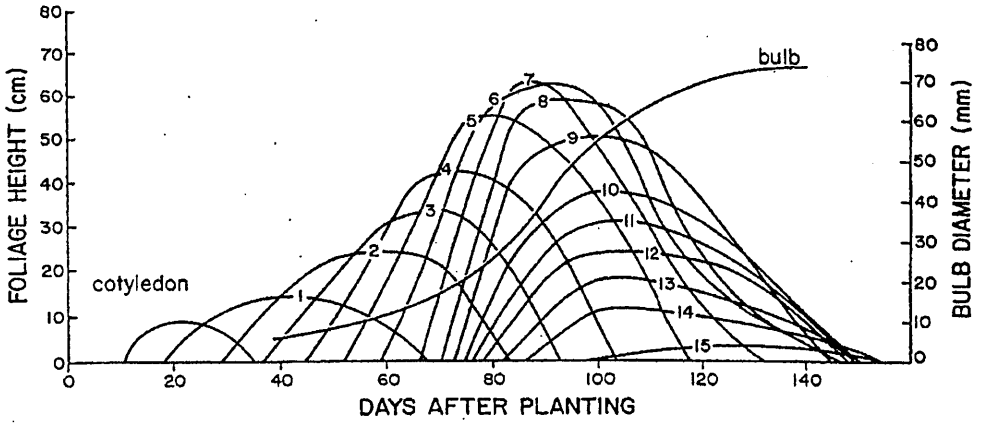

Figure 2. Growth of consecutive leaves of an onion throughout its development from seed emergence to dry bulb production. Numbers represent sequences of leaves formed (after Rubatzky and Yamaguchi, 1997).

At bulb maturity, the outermost 1 to 3 leaf sheaths become dry and thin, while the sheath tissues of the neck soften and lose turgidity. Consequently, the upper foliage falls over indicating maturity and harvest time (Brewster, 1994). At harvest, a rise in endogenous abscisic acid levels (ABA) in onion bulbs promotes bulb dormancy (Komochi, 1990).

The overall nutritional value of Alliums is relatively low, but they are eaten mainly for their flavour characteristics. The water content and calorie of a 148 g fresh onion bulb were *ca* 130 g and 60 kcals, respectively (Caunce and Son, 2003). Other nutrients in fresh onion bulb are shown in Table 1.

Table 1. Nutrient analysis for 148 g fresh onion bulb (after Caunce and Son, 2003).

Nutrient	Value	Nutrient	Value
Total fat	0.3 g	Total carbohydrate	14 g
Saturated fat	0 g	Dietary fibre	3 g
Cholesterol	0 g	Sugars	9 g
Vitamin A	0 mg	Protein	2 g
Vitamin E	0.31 mg	Sodium	5 mg
Folate	17 mg	Calcium	4 mg
Niacin	0.7 mg	Iron	0.3 mg

2.3 Preharvest Effects on Growth and Quality of Onions

2.3.1 Genetic component

Onions usually cross-fertilise naturally (Currah, 1990). Therefore, in an outcrossing population, variations among individual plants are found in appearance, yield and quality. Onion genotypes vary in total plant fresh and dry weights, bulb size, dry-matter content, water-soluble carbohydrate level, soluble sugar content, and pyruvate concentration (Darbyshire and Henry, 1979; Randle, 1992a; 1992b; Ashish *et al.*, 1995; Hamilton *et al.*, 1997; Boyhan *et al.*, 2001). Growth patterns for six red onion cultivars in the same environment were similar, but differed in rate of assimilate accumulation (Ashish *et al.*, 1995). Bulb size and fresh weight at harvest were higher in cultivars with high dry-matter contents. An investigation with 10 different onion cultivars indicated that the greater the proportion (%) bulb dry weight, the greater the non-structural water-soluble carbohydrates content as compared with low dry weight cultivars (Darbyshire and Henry, 1979). The greater proportional bulb dry weight was associated with increased fructan levels and reduction in bulb tissue water content (Darbyshire and Henry, 1979; Randle, 1992a). 'White Spanish' hybrid, 7.5% dry weight (low); 'Australian Brown', 12.5% dry weight (medium) and 'White Creole x Southport White Globe', 19.0% dry weight (high) were found to have fructan contents of *ca* 60, 100 and 155 mg g^{-1} fresh weight (fw), respectively (Darbyshire and Henry, 1979). Reports on the relationship between dry-matter and the reducing sugars fructose and glucose are, however, inconsistent. Contrary to the findings of Darbyshire and Henry (1979), Randle (1992a) reported poor association between non-structural water-soluble carbohydrates and reducing sugars. The high dry weight cultivars had lower fructose (*ca* 3 mg g^{-1} fw) and glucose (*ca* 0.5 mg g^{-1} fw) contents as compared with those for the medium (fructose, *ca* 13.5; glucose, *ca* 22 mg g^{-1} fw) and low (fructose, *ca* 23; glucose, *ca* 27 mg g^{-1} fw) dry weight cultivars. The sucrose content for the medium dry weight cultivar was the greatest (*ca* 22 mg g^{-1} fw) as compared with the high (*ca* 10.5 mg g^{-1} fw) and low (*ca* 6.5 mg g^{-1} fw) dry weight cultivars. The total carbohydrate content in the cell wall of onion bulb determined from cold alcohol-insoluble residues did not differ with genotype (Ng *et al.*, 1998). However, the amount of pectic galactose for instance, reduced from the inner tissue (17%) and outer tissue (12%) to the skin (1%). Uronic acid increased by 39% for

inner tissue, 48% for outer tissue to 59% for the dry skin. Similar values of rhamnose, fucose, arabinose, xylose and mannose were found.

Genotypic differences among onion cultivars with reference to flavour have been demonstrated (Schwimmer and Weston, 1961; Freeman and Whenham, 1975a; Randle, 1992b; Randle and Bussard, 1993a; Ashish *et al.*, 1995; Hamilton, 1997; Bacon *et al.*, 1999). These variations were ascribed to different patterns of S partitioning and assimilation in flavour pathways in specific genotypes (Randle, 1992b; Thomas and Parkin, 1994; Ashish *et al.*, 1995). Thomas and Parkin (1994) demonstrated differences in composition and levels of S-alk(en)yl-L-cysteine sulphoxides (ACSOs) in garlic (Fig. 3A) and onion (Fig. 3B) using HPLC.

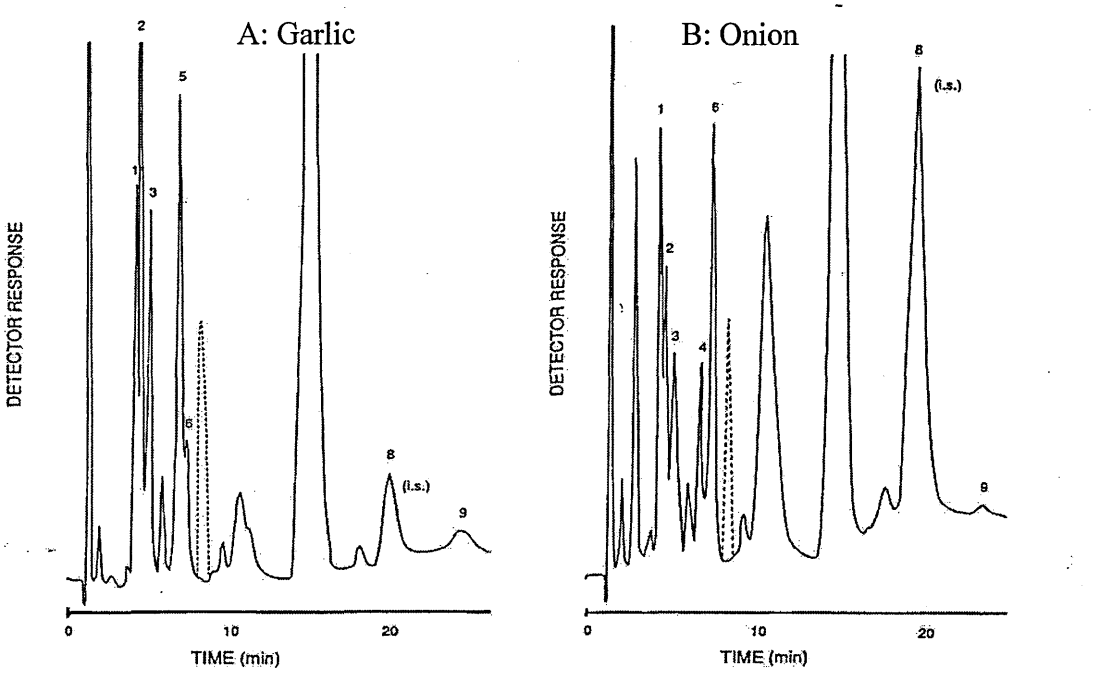

Figure 3. HPLC chromatograms of garlic (A) and onion (B) inner tissue extracted in methanol/chloroform/water (12:5:3 v/v/v) at -20°C. Peaks represents: 1, S-methyl-L-cysteine sulphoxide (MCSO); 2, cysteine; 3, glutamic acid; 4, glycine; 5, S-2-propenyl-L-cysteine sulphoxide (2-PRENCSO); 6, 1-PRENCSO; 7, 9-fluoroenylmethyl chloroformate (FMOC) alcohol derivatives; 8, ethyl cysteine (i.s.; internal standard) and 9, valine. Broken line represents S-propyl-L-cysteine sulphoxide (PCSO) (after Thomas and Parkin, 1994).

The rate of hydrolysis of individual flavour precursor ASCO compounds by the enzyme alliinase may depend on genotype and environment (Lancaster *et al.*, 1998).

These differences among genotypes led to classification by Schwimmer and Weston (1961) of onions into three groups of pungency levels. Onions with total pyruvic acid contents ranging from 2 to 4, 6 to 10 and 15 to 20 μmoles g^{-1} fw were classified as weak, intermediate and strong pungency, respectively. Recently, DEFRA (2001) proposed classification of enzymatically produced pyruvate concentration of between 0 and 5 μmoles g^{-1} fw as mild and between 6 and 10 μmoles g^{-1} fw as strong pungency.

2.3.2 Climate component

Climatic variables, especially temperature and light, are important determinants of onion growth and development when soil moisture and nutrients are not limiting (de Visser, 1990; Wheeler and Ellis, 1991; Brewster, 1990; 1994; 1997). High field temperatures enhance bulb swelling, dry-matter content, bulb yield and quality of the dry outer scale leaf (Currah and Proctor, 1990; de Visser, 1990; Rubatzky and Yamaguchi, 1997). Suggested temperature ranges for the juvenile stage of onion plant growth are 17° to 23°C (Robinson, 1971), 6° to 20°C (Brewster, 1997), 23° to 27°C (Rubatzky and Yamaguchi, 1997) and 13° to 24°C (Warade and Kadam, 1998) depending upon the cultivar and stage of phenological development. An increase in temperature from a range of 10° to 15°C to a range of 21° to 27°C during plant growth increased volatile sulphur compounds in macerated onion bulbs by 3.0-fold (Platenius and Knott, 1941). Increased temperature perhaps improved nutrient uptake and assimilation, thereby increasing ACSO synthesis.

Light influences onion bulb initiation and swelling (Brewster *et al.*, 1986; de Visser, 1990; Wheeler and Ellis, 1991; Brewster and Sutherland, 1993; Tei *et al.*, 1996a; 1996b). The duration, quality and intensity of light required by plants for growth and assimilate accumulation vary among genotypes (Garner and Allard, 1920). Bulb initiation in short-day, intermediate and long-day onion cultivars requires daylengths of 11 to 13 h, 13 to 14 h and >14 h, respectively (Currah and Proctor, 1990; Brewster, 1990; 1994; Rubatzky and Yamaguchi, 1997). A change in exposure of shallot seedlings from a 12 h daylength to >13 h daylength during bulbing increased bulb yields by 53% (Abbey and Fordham, 1998). Thus, emerging onion seedlings can be stimulated to produce bulbs when exposed to long photoperiod in a controlled

environment (Terabun, 1971).

Various mathematical regression and descriptive models have been used to describe the interactive effects of temperature and photoperiod on onion growth and dry-matter yield (Brewster *et al.*, 1986; Scaife *et al.*, 1987; de Visser, 1990; Wheeler and Ellis, 1991; Aikman and Scaife, 1993; Brewster and Sutherland, 1993; de Visser; 1994; Tei *et al.*, 1996a; 1996b). For a given photoperiod, the time of bulbing is dependent on temperature. Thus, an increase in temperature at a specific inductive photoperiod reduces time to bulbing. Conversely, a reduction in temperature at a specific inductive photoperiod delays time to bulbing.

Examples of mathematical regression models

a) Brewster *et al.* (1986) defined the mean net conversion efficiency of intercepted radiation to dry-matter (E) during bulbing as:

$$E = \frac{\Delta W}{\int_{t_1}^{t_2} Ipd_t}$$

Where ΔW = change in total dry-matter yield from onset of bulbing until harvest; I = irradiance at the top of the canopy; p = proportional radiant flux intercepted by leaf canopy; t_1 and t_2 = times of onset of bulbing and harvest, respectively; d_t = time difference between t_1 and t_2. Using this model the time from onset of bulbing to bulb maturation was found to be longer in autumn-sown crops. These autumn crops also had the highest yield due to their longer period of assimilate translocation into the bulbs as compared to spring-sown crops.

b) According to de Visser (1994), photothermal time (BULBSUM) to bulb formation and swelling from seedling emergence can be expressed as follows:

$$\int BULBSUM_i = \sum_i (TEFF_i * DAYFAC_i * RFRFAC_i)$$

where $BULBSUM_i$ = thermal time corrected for influence of daylength and red:far-red light ratio; $TEFF_i$ = temperature difference above a base of 6°C; $DAYFAC_i$ = factor correcting $BULBSUM_i$ for daylength; $RFRFAC_i$ = factor correcting $BULBSUM_i$ for the red:far-red light ratio. This model can be used to predict growth and bulb yield. The model might also be effective with appropriate modifications to

predict quality at harvest and during storage.

2.3.3 Edaphic variables and management practices

2.3.3.1 Soil factors

Among other influences the soil physical properties of structure and texture affect uptake and utilisation of water and mineral nutrients by roots (Beverly *et al.,* 1993; Rowell, 1996; Lambers *et al.*, 1998). Soil compaction increases soil strength and bulk density and thereby reduces soil porosity. These adverse soil structure effects reduce onion root growth (Chamen *et al.*, 1992). Onions are grown on a wide range of different substrates including light sand to heavy clay soils, peat and in hydroponics (Ko *et al.*, 1993; Brewster, 1994; Rubatzky and Yamaguchi, 1997; Hamilton *et al.*, 1998; Randle, 2000). Effects of variations in soil type on onion production and quality are inconsistent. Bulb fresh weight, bulb diameter and dry-matter content did not vary between clay and sandy loam soils at all sampling dates from 9 March to 21 April in Texas, USA (Patil *et al.*, 1995; Hamilton *et al.*, 1998). Bulb fresh weight and diameter ranged from 73.3 to 422.0 g and 4.9 to 10.1 cm, respectively, on sandy loam while the corresponding values for clay soil ranged from 63.8 to 387.2 g and 4.5 to 9.9 cm (Patil *et al.*, 1995). However, other workers reported growth improvement on clay as compared with sandy soil (Talha *et al.*, 1978; Hanson *et al.*, 2003). This difference may be due to variations in management, inherent soil factors and climate that were not measured and/or reported.

2.3.3.2 Irrigation regime

Clay and sandy soils vary in water retention capacities, which differentially affect onion production and quality (Talha *et al.*, 1978). The critical soil water content beyond which plant growth is impaired increases with an increase in soil particle size from <0.2 μm for clay to >2.0 mm for sand (Brady and Weil, 1996; Momose and Kasubuchi, 2002). The critical soil water potential measured in terms of specific energy for clay loam (Haplic Andosols) of particle density 2.44 g cm^{-3}, light clay (Haplic Acrisols) of particle density 2.70 g cm^{-3} and sand (Gleyic, Haplic Arenosols) of particle density 2.63 g cm^{-3} were -600, -100 and -6 J kg^{-1}, respectively (Momose and Kasubuchi, 2002). Generally, sandy soils have larger particles and less water storage capacity than clay. As a result, sands have low water storage capacity to

balance deficit irrigation. Canopy coverage and clove yield in garlic was reduced on sandy soil due to increased water-deficits (Hanson *et al.*, 2003). On clay soil, water deficits occurred but did not reduce growth and yield of onion (Talha *et al.*, 1978) and garlic (Hanson *et al.*, 2003). Under water-deficit conditions, garlic roots extracted water from a depth of >1.07 m in clay soil while in sandy soils, the roots did not reach further than 0.76 m (Hanson *et al.*, 2003).

Glenn (2000) explained that inadequate irrigation limits the size of plant root systems and their capacity to produce and transport cytokinin and nutrients to the shoots. Adverse effects of water deficits can reduce root physiological activities and total plant growth (Lambers *et al.*, 1998). Various soil water potential thresholds have been reported for maximising onion production. Klar *et al.* (1976), Abreu *et al.* (1980), Coelho *et al.* (1996), and Shock *et al.* (2000) reported soil water potential thresholds of -15 kPa, -10 kPa, -8.5 kPa and -17 kPa, respectively. In the Sudan Savannah ecological zone, weekly irrigation was found to greatly improve growth and bulb yield of a local onion cv. DS-79 compared to 40, 60 and 80 mm cumulative pan evaporation irrigation treatments (Hussaini and Amans, 2000). Irrigation at 40% available soil water capacity increased onion growth and bulb yield on clay while on sandy loam, irrigation at 20% available soil water capacity gave the best growth and yield (Talha *et al.*, 1978). Similarly, regular irrigation was shown elsewhere to increase onion bulb growth and flavour intensity (Gamiely *et al.*, 1991). Postharvest spoilage in terms of number of decayed or dried onion bulbs can be increased by preharvest water-deficits stress conditions (Narang and Dastane, 1972).

2.3.3.3. Soil nutrients

Clay soils typically have higher amounts of soil colloids, which are usually <2μm as compared to sand soils (Brady and Weil, 1996). These colloids are comprised of layered silicate clays, iron (Fe) and aluminium (Al) oxide clays, allophane and associated amorphous clays and humus. Consequently, clay soils have greater cation exchange capacity (CEC) than sandy soils. The CEC for sandy loam ranges between 2 and 12 cmole kg^{-1} and that for clay ranges between 4 and 60 cmole kg^{-1} (Rowell, 1996). CEC increases with an increase in soil pH. The greater the CEC of the soil, generally the greater the soil nutrient availability for root uptake (Brady and Weil,

1996; Rowell, 1996). Thus, the type of fertiliser applied and its effect on the soil pH and/or inherent soil nutrients can affect CEC and soil nutrient availability.

An increase in N application rate generally improves onion plant growth and bulb yield (Narang and Dastane, 1971; Brewster and Butler, 1989; Gamiely *et al.*, 1991; Sachdev *et al.*, 1991; Singh *et al.*, 1996; Hussaini and Amans, 2000; Randle, 2000). Such effects are also evident in other crops, including carrot (*Daucus carota* L.; Hochmuth *et al.*, 1999) and tomato (*Lycopersicon esculentum* Mill.; Sainju *et al.*, 2000). N deficiency early in growth delays onion bulb scale formation as compared with a high initial N rate, split application of N or late N deprivation (Brewster and Butler, 1989). This finding agrees with work of Buwalda and Freeman (1987) where the greatest positive effect of N on relative growth rate of onion occurred at early stages of growth. Application of 70 kg N ha^{-1} increased onion bulb yield by 2.41-fold over no N addition treatment (Narang and Dastane, 1971). The association between bulb yield (Y-axis) and rate of N application (X-axis) could be described by the quadratic function $Y = \alpha + \beta_1 X_2 + \beta_2 X_2^2$ (Narang and Dastane, 1971). Thus, yield levelled off or dropped after an optimum N rate. The form of this function (i.e. constants in the equation) depends on genotype and/or environment factors. A levelling off or reduction in yield with continuous high N application, especially late in growth, was attributed to an increase in soil electrolyte conductivity (EC) value (Souma and Iwabuchi, 1981). An increase in the EC value reflects high salinity level of the soil (Rowell, 1996).

In a pot experiment, N fertiliser application rate of 0.22 g l^{-1} increased bulb fresh weight and firmness. However, these growth characteristics were reduced by *ca* 18% upon increasing N to between 0.41 and 0.97 g l^{-1} (Randle, 2000). An increase in N up to 0.60 g l^{-1} increased the concentration of the onion flavour precursors MCSO (2.63 mg g^{-1}) and PCSO (0.31 mg g^{-1} fw), but reduced 1-PRENCSO from 0.76 mg g^{-1} fw at 0.22 g N l^{-1} to 0.73 mg g^{-1} fw (Randle, 2000). As N rate was further increased from 0.60 to 0.97 g l^{-1}, the concentration of the flavour precursors reduced.

The source of N fertiliser affects onion growth and yield (El-Bana *et al.*, 1988; Gamiely *et al.*, 1991). Generally, nitrate-nitrogen (NO_3-N) alone increases onion

plant growth, bulb yield and flavour more than application of ammonia-nitrogen (NH_4-N) alone. An increase in the ratio of NO_3-N:NH_4-N from 1:3 to 3:1 increased the fresh weights of the leaves and roots and the bulb dry weight of onion cv. Granex 33 from 152 to 218 g plant^{-1}, 10.7 to 28.0 g plant^{-1} and 7.4 to 8.3%, respectively (Gamiely *et al.*, 1991). The fresh weight and flavour of the bulbs were not affected by increased NO_3-N:NH_4-N ratio from 1:3 to 3:1. N rate and source did not affect sugar content of the bulbs.

Responses of onions to S fertilisation are widely studied and well understood. Interactions of onion genotype by S for plant growth and quality were reported by Randle (1992a; 1992b), Randle and Bussard (1993a), Randle *et al.* (1995) and Hamilton *et al.* (1997). Onion genotypes varied markedly in their degree of response to S fertilisation. Among 62 bulb onion genotypes, changes in flavour and percentage dry weight following an increase in S application from 0.1 meq S l^{-1} (32 mg S l^{-1}) to 4 meq S l^{-1} (128 mg S l^{-1}) ranged between 1.1- for entry (genotype code) JP 7 to 3.0-fold for entry PI 168960 and 6.8- for entry JP 19 to 17.3-fold for entry PI 172701, respectively (Randle, 1992a). A minor degree of S deficiency may not affect crop yield but can markedly reduce crop quality (Hawkesford, 2000) including onion flavour (Freeman and Mossadeghi, 1970; Randle *et al.*, 1994; 1995; Randle, 1997b; Hamilton *et al.*, 1997; 1998). In Athens (Georgia, USA) the S concentration of onion leaves increased from 0.55% (on dry weight basis) in November to 0.8% in January with an increase in S fertilisation up to the seedling stage, after which leaf S concentration reduced to 0.2% at harvest in June (Randle *et al.*, 1993). The reduction in leaf S concentration with growth advancement was explained by re-mobilisation of S into developing bulbs during their swelling and maturation (Lancaster *et al.*, 1986).

The application of S fertiliser at all levels from 0 to 170 kg S ha^{-1} did not affect the fresh weight of sound bulbs and the proportion of cumulative losses due to sprouting after 6.5 months storage under ambient conditions (Narang and Dastane, 1972). However, the 170 kg S ha^{-1} treatment increased bulb decay and/or drying by 1.32-fold as compared with no S addition.

A synergistic association exists between plant absorbed N and S in that the activity of

N reductase is dependent on S supply while that of ATP sulphurylase is dependent on N supply during synthesis of cysteine (Reuveny *et al.*, 1980; Fox and Blair, 1986; Singh *et al.*, 1996). Thus, variation in the N:S ratio can affect onion growth, flavour quality and degree of resistance to disease causing pathogens. Reductions in crop quality due to S deficiency can be ascribed to imbalances in the N:S ratio (Zhao *et al.*, 1996), the optimum of which varies with the crop species. S deficiency is evidenced as low plant tissue S concentration in addition to high soluble N pools, which include nitrates and amides. An N:S ratio <15 for wheat grains for instance, suggested excessive application of S fertiliser over that required by the available N for protein synthesis (Byers and Bolton, 1979). Conversely, an N:S ratio of >15 implied excessive N fertiliser over that required by the available S for protein synthesis. Such a relationship between N versus S has not been established in onions. N and S applications increased plant growth and bulb yield on a S-deficient sandy soil (Sachdev *et al.*, 1991). An increase in application of S fertiliser alone from 0 to 80 kg S ha^{-1} did not affect bulb yield of onion cv. Pusa Red (Singh *et al.*, 1996). However, increases in amounts of N from 0 to 120 kg ha^{-1} and S from 0 to 40 kg ha^{-1} increased bulb yield by 1.53-fold as compared with no fertiliser addition. Bulb yield rose by 1.05-fold as N and S applications were increased from 40 and 120 kg ha^{-1}, respectively, to 80 and 180 kg ha^{-1}. For adequately fertilised (N, P, K and S) soils in the Columbia Basin (USA), the total onion leaf S concentration at the 3- to 8-leaf stage was between 0.5 and 0.8% on dry weight basis and for harvested bulbs, between 0.3 and 0.6% (Sullivan *et al.*, 2001). The N:S ratio varied between 3-5:1, which is deemed safe. However, Smittle *et al.* (1979) suggested that cultural practices which reduce S but increase N supply to plants may produce less pungent onions. Further work is required to test this hypothesis on different soil types.

Conventional determinants of *Allium* flavour include the lachrymatory factor and thiosulphinate concentrations (Freeman and Mossadeghi, 1970; Block, 1992; Block *et al.*, 1992). The ratio of ACSO:pyruvate:thiosulphinate upon onion tissue disruption is 2:2:1 (Freeman and McBreen, 1973). Thus, onion flavour including those of the other Alliums can be estimated by determination of pyruvic acid concentration, which is known to be relatively stable (Schwimmer and Weston, 1961; Freeman and Whenham, 1975a; Thomas and Parkin, 1994). Thiosulphinate and pyruvic acid

concentrations typically increase with increasing rate of S application (Freeman and Mossadeghi, 1970; Randle, 1992a; Randle and Bussard, 1993a; Randle *et al.*, 1993; 1994; 1995; 1999; Hamilton *et al.*, 1997; 1998). Application of 3 meq S l^{-1} (96 mg S l^{-1}) increased the pyruvate concentration of spring onion cv. White Lisbon to 9.2 µmole ml^{-1} compared with 0.58 µmole ml^{-1} for no S application (Freeman and Mossadeghi, 1970). Conversely, soluble sugar contents in 34 out of 69 onion bulb cultivars were reduced by 12% with the application of 4 meq S l^{-1} (128 mg S l^{-1}) as compared with a 0.1 meq S l^{-1} treatment (32 mg S l^{-1}; Randle, 1992a; Randle and Bussard, 1993a).

Apart from N and S, P is also important in onion production (Narang and Dastane, 1971; Sullivan *et al.*, 2001). P improves root growth and bulb size, and also hastens bulb maturation (Narang and Dastane, 1971; Tseng, 1972; Sullivan *et al.*, 2001). Availability and uptake of soil P by onion roots were enhanced by root colonisation by the vesicular-arbuscular mycorrhiza (VAM) fungi *Glomus deserticola* and *G. Mosseae* (Poss *et al.*, 1985; Currah and Proctor, 1990). This response was evident even in an unfavourable soil environment such as at a high salinity level of 8.8 dS m^{-1} and under water-deficit stress conditions (Poss *et al.*, 1985). Other *Glomus* spp. of importance in P uptake include *G. occultum, G. manihotis* and *G. intraradices* (Anonymous, 1997).

Although some workers have reported no effect of K on onion growth (Narang and Dastane; 1971; Hassan and Ayoub, 1978; Singh, 1978; Morales *et al.*, 1992), it is possible under certain conditions that K can improve onion quality (Tseng, 1972). Onion response to micronutrients such as Zn and B is greatest at the bulb growth stage, normally from 90 days after sowing (Sullivan *et al.*, 2001). However, the application of micronutrients is usually not recommended.

2.4 Effects of Postharvest Treatments on Onion Storage Ability and Flavour

2.4.1 Textural changes

Flesh hardness of intact onions was associated with pungency and storage ability

(Onionz, 2002). For mild, short storage onion varieties flesh hardness was between 5 and 6 kg. In contrast, flesh hardness of highly pungent and long storage onions was between 11 and 17 kg. Leaf curvature in intact green onion (*Allium cepa* x *A. fistulosum*) did not change after 21 days of storage at 0°C as compared with storage at 5° or 10°C, which increased leaf curvature due to textural changes (Hong *et al.*, 2000). The increase in leaf curvature at ≥5°C was recorded for green onions stored in air alone or air plus 9% CO_2, but not in 0.1% O_2 alone or 0.1% O_2 plus 9% CO_2 CA treatment.

Minimal processing techniques such as peeling, slicing, dicing and wedging of fresh horticultural produce cause substantial injury which adversely affects normal metabolic function and increases spoilage (Shewfelt, 1987; Kader, 1992; Saltveit, 1999). Textural changes and browning, for example, are common in minimally processed onions (Blanchard *et al.*, 1996; Toivonen, 1997; Hong *et al.*, 2000) and other fresh vegetables (King and Bolin, 1989; Guerzoni *et al.*, 1996; Lopez-Galvez *et al.*, 1996). Diced onions stored in a controlled atmosphere of 2% O_2 and 5% CO_2 become less firm within 10 days at 1°C with a concomitant increase in electrolyte leakage (Khatoon and Hakim, 1999). The cell walls of onions are comprised mainly of pectic polysaccharides (Ng *et al.*, 1998). β-galactosidase and exo-polygalacturonase enzyme activities have been implicated in the hydrolysis of pectic compounds leading to textural changes in wounded tissues (King and Bolin, 1989). Howard *et al.* (1994) associated a reduction in soluble phenol production in diced onion after 6 days of storage to an increase in lignin formation or other polymers during the wound healing process, leading to an increase in firmness. In contrast, cell wall carbohydrates of intact onion stored at 0°C and 60-65% RH remained unchanged after 6 months (Ng *et al.*, 1998).

2.4.2. Browning disorder

Polyphenol oxidase (PPO) reacts with endogenous substrates such as o-dihydroxy phenols to produce quinones in wounded fresh tissues (King and Bolin, 1989; Amiot *et al*, 1997). Further enzymatic action on quinones results in tissue browning. High levels of phenylalanine lyase (PAL), ethylene (Lopez-Galvez *et al.*, 1996), oxidoreductases and peroxidases (Amiot *et al.*, 1997; Toivonen, 1997) enhance

browning. Genetic factors such as the rate of quinone formation, oxidase enzyme activity and environment variables such as temperature all influence the rate of tissue browning (Lopez-Galvez *et al.*, 1996; Amiot *et al.*, 1997). Post-cooking browning in diced onion was increased in association with an increase in soluble phenol concentration in bulbs during storage (Blanchard *et al.*, 1996), an effect that may vary with genotype. Camelo and Cantwell (1999) demonstrated that browning in diced onion increased with an increase in storage temperature from 0° to 10°C.

2.4.3 Postharvest respiration

Storage at either 13° or 20°C for 8 weeks increased the rate of respiration of intact bulbs to *ca* 230 μg CO_2 g^{-1} h^{-1} as compared with a respiration rate of *ca* 63 μg CO_2 g^{-1} h^{-1} at 1°C (Yoo *et al.*, 1997). The beginning of post-dormancy activities was marked by a rise in respiration to >400 μg CO_2 g^{-1} h^{-1} after 12 weeks at 27°C.

Rapid diffusion of O_2 into onion tissue that had been diced enhanced respiration (Blanchard *et al.*, 1996), which possibly facilitated the wound healing process. However, high rates of respiration can deplete sugar levels and other food reserves and thereby adversely affect flavour and other quality attributes (Carlin *et al.*, 1990). An O_2 concentration of between 0.1 and 2% alone or in combination with CO_2 treatment between 7 and 10% in a controlled atmosphere (CA) system at 0° to 5°C increased the potential storage duration by better maintaining visual quality and reducing respiration rate in cut onions (Day, 1989; Blanchard *et al.*, 1996; Khatoon and Hakim, 1999; Hong *et al.*, 2000). Reduction in respiration rate due to CA conditions tends to reduce energy supply to wounded onion tissue (Hong *et al.*, 2000). The reduction in energy supply can in turn affect enzymatic steps for colour changes in cut onions, and extend bulb dormancy. However, anaerobic respiration may ensue when CO_2/O_2 ratio increases for cut onions as has been reported for strawberry fruit (Larsen and Watkins, 1995). Anaerobiosis leads to the production of off-flavour compounds, such as acetaldehyde, ethanol and methanethiol, and increased spoilage. Clay absorbent made up of quartz, feldspar, amphibole and traces of analcime and potassium permanganate gas absorbent may reduce these off-flavours (King and Bolin, 1989; Howard *et al.*, 1994; Toivonen, 1997).

Preharvest application of the ethylene releaser ethephon, increases seed yield and bulb size, but reduces foliage growth and oligosaccharides and total sugar content in some onion cultivars (Ostrzycka and Gorecki, 1991). Endogenous ethylene is also known to moderate ABA role in enhancing bulb dormancy (Komochi, 1990).

Wounding and anaerobic respiration in the presence of high CO_2 concentration stimulates ethylene biosynthesis via increased ACC (1-aminocyclopropane-1-carboxylate) oxidase activity (Yu and Yang, 1980; Howard *et al.*, 1994; Mathooko *et al.*, 1995; Mathooko, 1996). ACC oxidase converts ACC into the phytohormone ethylene, which promotes tissue breakdown. However, the mechanism(s) involved in CO_2 induction of ethylene production is not understood (Mathooko, 1996). Ethylene concentration in the headspace of diced onions wrapped in polyethylene bags increased to 3.0 µl l^{-1} after 3 days of storage at 2°C, but subsequently reduced to 2.2 µl l^{-1} after 6 days and 0.7 µl l^{-1} after 10 days (Howard *et al.*, 1994). The early high concentration of ethylene was attributed to the tissue wounding response. Thus, reductions in ethylene and soluble phenolic concentrations in the headspace of stored minimally processed onions after 3 and 6 days of storage at 2°C, respectively, can be attributed to lignin formation or other wound healing polymers. The inclusion of potassium permanganate as a scrubber markedly reduced ethylene build-up from 0.1 µl l^{-1}after day one to 0.0 µl l^{-1}.

2.4.4 Microbial load

Microbial populations on minimally processed vegetables including onions can be prolific (King and Bolin, 1989; Watada and Qi, 1999; Howard *et al.*, 1994; Blanchard *et al.*, 1996; Khatoon and Hakim, 1999). Rapid microbial growth can be ascribed to ease of entry in tissues and to leakage of cellular fluids rich in nutrients (King and Bolin, 1989). An increase in populations of fungi from 10^4 to 10^8 colony forming units (CFU) g^{-1} reduced quality of diced onion stored at 4°C (Blanchard *et al.*, 1996). For bacterial growth, workers generally found little or no association between total bacterial count and shelf life (King *et al.*, 1976; Nguyen-The, 1991; Zagory, 1999). Bacterial growth was reported to stabilise at 10^8 bacteria g^{-1} during storage without affecting onion sensory quality (Nguyen-The, 1991). Similarly, Howard *et al.* (1994) did not find visible signs of decay in diced onions even though bacteria count rose to

10^6 g^{-1} after 10 days of storage at 2°C. Nonetheless, microbial exudates and dead microbial cells may contribute to discolouration and loss of translucence in fresh produce (King and Bolin, 1989).

Volatile organo-S compounds derived from degradation of ACSO in Alliums have anti-microbial properties (Block, 1992; Kyung *et al.*, 2002). For instance, the two main anti-microbial compounds of garlic are methyl methane thiosulphinate and allyl 2-propene thiosulphinate (allicin), which are formed during tissue disruption from methyl- and allyl- derivatives of ACSO (Kyung *et al.*, 2002) (see section 2.3.5.1 for further explanation on ACSOs). Phenols are also known to inhibit growth of some micro-organisms in onions (Saltveit, 1997). The scale leaves of intact brown or red skin onions contain large proportion of the phenolics protocatechuic acid and catechol, which protect the bulb against disease pathogens compared to white skin onions (Walker, 1925; Walker and Stahmann, 1955). Preformed phytoalexins belonging to the class cyclic dione, namely 5-octyl-cyclopenta 5-1, 3-dione and hexylcyclopenta-1, 3-dione are also found in Alliums (Dmitriev *et al.*, 1990; Tverskoy *et al.*, 1991). A CA gas composition of 2% O_2 plus 10% CO_2 at 4°C reduced microbial load in diced onions (Blanchard *et al.*, 1996).

2.4.5 Compositional changes in stored onions

2.4.5.1 Flavour compounds and flavour

Alliums are comprised of 24 γ-glutamyl peptides, which are major N and S storage compounds (Lancaster and Shaw, 1991; Block, 1992; Block et al., 1992). Two of these peptides i.e. γ-glutamyl trans-(+)-S-(2-propenyl)-L-cysteine sulphoxide (γ-glutamyl PRENCSO) and S-2-carboxypropyl glutathione (2CPGTH) are intermediates in the biosynthetic pathway to flavour precursors in onions (Block, 1992; *Appendix* IV). Peptides are formed by joining an α-carboxyl group of one amino acid to the α-amino group of another amino acid by a peptide or an amide bond (Stryer, 1998). Alliums generally contain non-protein sulphur amino acid secondary metabolites that give the characteristic flavours and aromas. These metabolites form between 1 and 5% of total dry weight (Block, 1992).

Inter and intra-specific variations in flavour quality and intensity among *Allium* spp.

have been reported (Freeman and McBreen, 1973; Freeman and Whenham, 1975a; Lancaster and Kelly, 1983; Block *et al.*, 1992; Randle, 1992a; 1992b; Thomas and Parkin, 1994; Randle *et al.*, 1995; Hamilton *et al.*, 1997; Yoo and Pike, 1998; Bacon *et al.*, 1999; Debaene *et al.*, 1999; Kopsell and Randle, 1997; Kopsell *et al.*, 1999; Randle *et al.*, 1999). Flavours and aromas of Alliums are derived from the ACSOs S-methyl- (MCSO), S-propyl- (PCSO), S-1-propenyl- (S-1-PRENCSO) and S-2-propenyl-(S-2-PRENCSO)-L-cysteine sulphoxides (*Appendix* IV). All of these ACSOs are detected in onions except S-2-PRENCSO, which is found mostly in garlic. The most important ACSO in onion is S-1-PRENCSO (Schwimmer, 1967; Lancaster and Boland, 1990; Block, 1992; Block *et al.*, 1992), which is responsible for the sensory attributes experienced when onion is eaten or wounded (Fig. 4; Schwimmer, 1967).

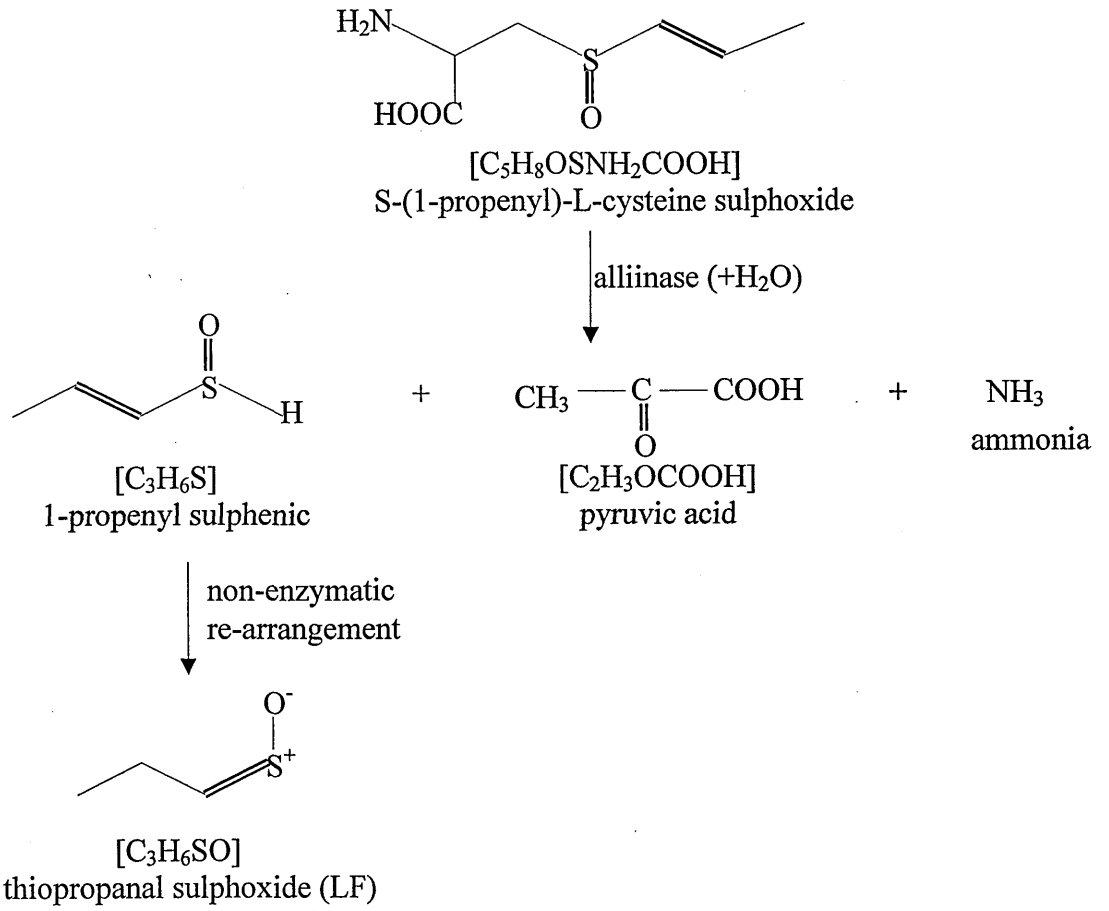

Figure 4. Schematic diagram of onion flavour biosynthesis during tissue disruption.

The hydrolytic enzyme alliinase is released from the vacuole into the cytoplasm upon disruption of onion tissue (Lancaster and Kelly, 1998). In the cytoplasm, flavour

precursors are hydrolysed producing both flavour and non-flavour compounds along with pyruvic acid and ammonia (Fig. 4; Freeman and Whenham, 1975b; 1975c; Block, 1992; Thomas and Parkin, 1994; Lancaster and Kelly, 1998). Pyruvic acid produced in this reaction is stable. Another product sulphenic acid quickly re-arranges non-enzymatically into a highly volatile lachrymatory factor thiopropanal sulphoxide (LF; a tear stimulant), which vaporises readily.

The flavour and aromas of green onions are derived from the thiosulphinates 1-propanesulphonothioic acid S-(-Z)-propenyl ester, 1-propane sulphinothioic acid S-1-propyl ester and methane sulphinothioic acid S-(Z)-propenyl ester (Block *et al.*, 1992). The mass ratio of S-1-PRENCSO:MCSO:PCSO was estimated to be 80:18:2 (Freeman and Whenham, 1975a). Thomas and Parkin (1994) reported S-2-PRENCSO:MCSO of 84:16 (w/w) for garlic and S-1-PRENCSO:MCSO of 86:14 (w/w) for onion.

Concentrations of intermediate peptides in onion flavour biosynthesis, the ACSOs and flavour intensity alter during storage (Lewis *et al.*, 1977; Debaene *et al.*, 1999; Kopsell *et al.*, 1999; Randle *et al.*, 1999; Benkeblia, 2000; Hong *et al.*, 2000). However, relationships among these various compounds during onion storage are inconsistent. The overall change in total ACSO among seven onion cultivars differed during 4 months of storage (Kopsell *et al.*, 1999). MCSO concentration reduced in the seven cultivars after 6 months of storage. A linear reduction in the concentration of γ-glutamyl PRENCSO corresponded to a linear increase in concentration of S-1-PRENCSO. The concentration of PCSO was generally low in all the cultivars during storage. Lancaster and Shaw (1991) showed that during bulbing the amounts of γ-glutamyl PRENCSO and 2-CPGTH glutathione increased by 2.5% of total dry weight while total ASCO increased 2.0-fold. The levels of these compounds were maintained during storage at 10°C but dropped by 30 to 50% after 6 months when dormancy was broken.

For diced onions in polyethylene wrapper, the total sulphur volatiles including flavour compounds was higher in bags that did not contain potassium permanganate absorbent (Howard *et al.*, 1994). However, there was an overall decline in total

sulphur volatiles by 58% after 10 days of storage at 2°C across the treatments either with or without potassium permanganate gas absorbent.

The standard measure for *Allium* flavour pyruvic acid concentration (Schwimmer and Weston, 1961; Randle and Bussard, 1993b) varied with both cultivar and storage duration (Kopsell and Randle, 1997). For short-day flowering cultivars, the direction of change in concentration of enzymatically-produced pyruvate (EPY; i.e. total pyruvate – background pyruvate) was inconsistent. Thus, EPY either increased or decreased with increasing storage duration. In contrast, EPY reduced with duration of storage of intermediate- and long-day flowering cultivars. The relationship between EPY (Y-axis) and total ASCO (X-axis) is poor ($Y = 20.4 + 1.26X$; $r^2 = 0.30$; Randle *et al.*, 1995; Kopsell *et al.*, 1999). However, a linear association between EPY (Y-axis) and PRENCSO (X-axis) was strong and significant ($Y = 1.7 + 1.8X$; $r^2 = 0.81$; $P<0.05$; Randle *et al.*, 1995). Nonetheless, other experimental evidence suggests a strong linear correlation ($Y = -2.76 + 1.12X$; $r^2 = 0.861$) between EPY (Y-axis) versus total ASCOs (X-axis; Bacon *et al.*, 1999). The lack of correlation in the work of Kopsell *et al.* (1999) as compared with that of Bacon *et al.* (1999) might be explained by differences in experimental treatments. These experimental treatments were variations in onion genotype versus storage duration (Kopsell *et al.*, 1999) and variations in genotype versus tissue type (Bacon *et al.*, 1999).

Pyruvic acid concentration in diced onion kept in a semi-permeable modified atmosphere polyethylene bags package at 4°C was reduced by 30 to 40% over 14 days of storage as compared with intact onion (Blanchard *et al.*, 1996). This reduction in flavour could be ascribed to escape of low molecular weight aromatic sulphur volatiles through film wrappers and/or a metabolic reactions leading to low amounts of flavour compounds (Howard *et al.*, 1994). Endogenous alliinase activity is reduced by prolonged storage at low temperature of ≤4°C (Lewis *et al.*, 1977; Benkeblia, 2000). Aromatic volatiles accumulate in the headspace of minimally processed onions wrapped in polyethylene film. Similarly, non-flavour volatiles such as acetyaldehyde, methanethiol and propanethiol also accumulate and can taint the flavour of wrapped diced onions (Larsen and Watkins, 1995; Toivonen, 1997).

2.4.5.2 Sugar content, carbohydrate composition and water loss

The proportion of the sugars sucrose, glucose and fructose relative to pungency level as indicated by pyruvic acid concentration determine onion sweetness (MKS, 2002). High level of pungency can mask high levels of sugars and therefore, sweetness of onion. Low pungency plus low levels of sugars give a bland onion. While sweet onion is characterised by low pungency and high levels of sugars, especially high fructose and glucose concentrations relative to sucrose. Proportional (%) fresh weight and carbohydrate composition of intact onions did not change after 6 months of storage at 0° and 20°C (Ng *et al.*, 1998). Usually, short storage onion cultivars such as 'Grano de Oro' have high sucrose, glucose and fructose contents, but low contents of fructooligosaccharide (FOS) and hardly any fructans due to hydrolysis of the latter to free fructose during storage (Jaime *et al.*, 2001; Kopsell and Randle, 1999). Conversely, onion cultivars that store for long such as 'Sturon' and 'Hysam' usually have less sucrose, glucose and fructose contents, but higher contents of fructans and lowest polymerised FOS as the major oligomer.

Prior to storage, fructan concentration was high but reduced with prolonged storage duration (Kopsell and Randle, 1997). The sucrose concentration of diced onion was greatest when kept at 2% O_2 plus 10% CO_2 and 4°C (Blanchard *et al.*, 1996). Although sucrose level reduced after 10 days of storage, the reduction was not so steep as compared with either air storage or a 2% O_2 treatment. Concentrations of the reducing sugars glucose and fructose and of total sugars were generally maintained throughout the storage period of 14 days at 4°C. While the reducing sugars declined from an average of 20.5 to 18.4 mg g^{-1} fw for glucose and from 18.4 to 15.8 mg g^{-1} fw for fructose, sucrose levels increased from 5.2 to 7.0 mg g^{-1} fw after 24 hr storage at 2°C (Howard *et al.*, 1994). Howard *et al.* (1994) explained that the increase in sucrose level might have been due to low temperature sweetening when onions were diced at 23°C and transferred to a 2°C storage unit as previously shown for other crops by Rees *et al.* (1981).

Loss in bulb fresh weight during storage can be attributed to moisture escape through the neck region of cured bulbs (Komochi, 1990) and can vary with cultivar (Kopsell and Randle, 1997). Kopsell and Randle (1997) reported pre-storage weight loss of

2.1% in 'Dehydrator #3' and 4.2% in 'Granex #33' after one month of storage. Loss of plant tissue water content adversely affects cell turgor and texture (Saltveit, 1997). Desiccation of minimally processed onion is faster than of intact bulbs due to the extensive wounds, removal of protective skin and exposure of internal tissues (Camelo and Cantwell, 1999; Khatoon and Hakim, 1999; Watada and Qi, 1999). Packaging of minimally processed onions in polyethylene bags and wound healing protect cells from desiccation and can minimise spoilage (Saltveit, 1997). Applications of edible coatings to cut or wounded onions can also help maintain high relative humidity (Watada and Qi, 1999) to minimise water loss.

2.4.5.3 Onion skin thickness and skin loss

Removal of dry scale leaves from stored onion bulbs in addition to damage to the stem base, but not low numbers of dry scale leaves hasten breaking of dormancy (Fustos, 1997). Retention of onion skin is dependent on its physical condition, number of dry scale leaves and on postharvest treatment of bulbs (Currah and Proctor, 1990). An increase in RH from 16 to 95% increased the moisture content of onion skins from 28% to 33.6% for cv. Hysam and from 2.1% to 33.3% for cv. Crossbow after 3 days (Hole *et al.*, 2000). The increased skin moisture content due to the 95% RH treatment increased skin thickness and burst (breaking) pressure (Hole *et al.*, 2000). Consequently, skin resistance to breakage was improved 2.0-fold as compared with storage at 16% RH. This effect suggests that onion bulb storage at high relative humidity is required to minimise skin peeling and to improve storage ability. Skin cracking is less in bulbs with skin thickness >0.04 mm and a tensile strength >3 kg (Currah and Proctor, 1990).

2.5 Application of Electronic Nose Technology for Discrimination of Headspace Volatiles

2.5.1 Odour perception by human nose

Flavours consist of various volatile and non-volatile chemical compounds (Taylor, 1998). Odour perception by the human nose is conferred by volatiles of polar molecules weighing between 20 and 300 Da (Bartlett *et al.*, 1997). Partitioning of flavours into the gaseous phase is dependent on time and the physico-chemical

properties of the substrate and the dilution medium (Taylor, 1998). The human nose detects low concentrations of odours at <1 nl l^{-1}. However, perception is constrained by factors such as environment, psychology (e.g. attention span, expectations and anticipations), education level, age, health and genetics (Manley, 1993). Sensory panellists are subject to fatigue and inconsistency, making sensory results generally unreliable as compared with analytical tests (Giese, 2000). Nonetheless, sensory appraisal remains the ultimate test for fresh produce flavour and other related quality indices (Mattheis and Fellman, 1999) including those for Alliums.

2.5.2 The concept of electronic nose technology

The history of electronic noses (E-noses) dates back about 39 years from mechanical (Tanyolac and Eaton, 1950) to electronic devices from 1964 (Wilkens and Hartman, 1964). Recently, the E-nose technology has generated lots of interest regarding its potential for odour discrimination in a wide range of disciplines including agriculture and medicine. Commercialisation of the E-nose technology in the food industry has not been exploited (Magan, 2001) due to limitations explained in later sections. Persaud and Dodd (1982) were the first to introduce the concept of modern E-noses. They proposed an E-nose built on an array of non-selective sensors with a corresponding pattern recognition system. Since then, many investigations and technical progress have been made in the development of E-noses using various sensor materials.

Analytical instruments such as high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) can be used to determine individual components of headspace flavour volatiles (Block, 1992; Giese, 2000). Like sensory tests, most analytical determinations of flavour are time-consuming, costly and require long periods of training. Simplified techniques have been developed for onion quality discrimination. These include determination of thiosulphinates using spectrophotometers (Freeman and McBreen, 1973), the N-ethylmaleimide reaction for thiosulphinates (Thomas *et al.*, 1992) and an automated HPLC system (Yoo and Pike, 1999) connected to an autosampler. Such analytical systems measure colour changes depending on sensor type. These techniques are improvement over traditional

analytical methods i.e. GC-MS and HPLC, but they can be slow and costly. In contrast, the relatively novel E-nose technology is a semi-quantitative technique that can 'fingerprint' aroma. The E-nose also offers safety, ease of use, speed and cost-efficiency. It also allows automation of odour evaluation (Payne, 1998; Giese, 2000).

The design and principle of operation of the E-nose have been likened to the human olfaction system (Bartlett *et al.*, 1997; Gopel *et al.*, 1998; Payne, 1998; Canhoto and Magan, 2003). The human olfaction system is comprised of three major parts, namely an array of olfactory receptor neurons at the top of the nose for sensing, the olfactory bulb for signal processing and the central nervous system (brain) for signal recognition (Gopel *et al.*, 1998; Payne, 1998; Canhoto and Magan, 2003). E-noses also have three main components, namely a sample station, which conditions headspace volatiles, sensor unit that accommodates the sensor array and a processing unit for analysing sensor responses using various pattern recognition engines. The principles of E-nose operation involve signal (odour) input, signal transduction and odour recognition (Fig. 5; Gopel *et al.*, 1998; Ziegler *et al.*, 1998; Gardner and Bartlett, 1999; Giese, 2000). Sensor properties changes during interaction with sample odours.

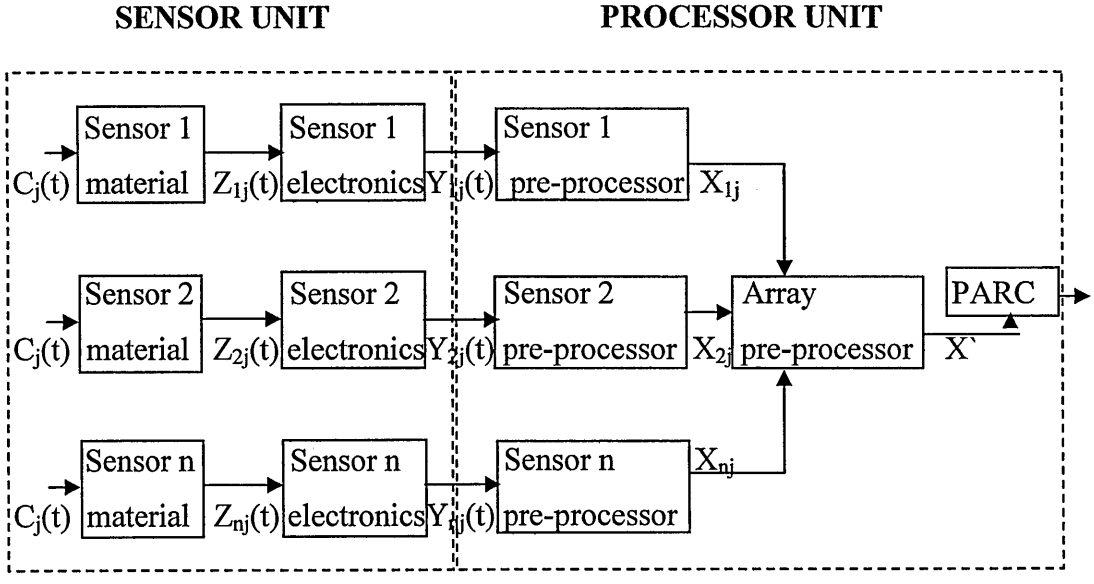

Figure 5. Schematic diagram showing the general principle of operation of an electronic nose in a modular system. An input signal odour j of a concentration, C at time t was translated into an output electronic signal X` upon interaction with n (total number of E-nose sensors) sensors. X` is then processed using pattern recognition (PARC) methods (after SRL, 2002).

The resultant signals are processed and interpreted using pattern recognition engines such as Principle Component Analysis (PCA), Artificial Neural Network (ANN), Discriminant Function Analysis (DFA) and Cluster Analysis (Bartlett and Gardner, 1997). A typical E-nose is comprised of a modular system consisting of sensor systems made up of various transducers (Ziegler *et al.*, 1998). The transducers facilitate maximisation of correlations in odour discrimination.

Chemical stability and efficient partitioning of odour molecules into the gaseous headspace phase are required. E-nose sensor response and efficient odour discrimination is thereafter dependent on the partition coefficient (K) for equilibrium distribution of odours between the gas phase and the sorbent phase of a sensor element (Grate *et al.*, 1997). An increase in the value of K (= C_s/C_v; where C_s is concentration of sample in the sorbent and C_v is concentration of sample in the gas phase) increases the strength of absorption of gas molecules into the sensor materials.

The Mahalanobis distance (D^2) model can be used to determine any differences between odour fingerprints (Mahalanobis, 1941; Morrison, 1976; Gnanadesikan, 1977; Mark and Tunnell, 1985; Shah and Gemperline, 1989). D^2 is determined from Principal Component (PC) scores and assumes a multivariate normal distribution for a given population. Mathematically, D^2 is defined as: $D_i^2 = [(x_i - \mu)\Sigma^{-1}(x_i - \mu)`]$; where . x_i = distance from ith sample, μ = class centroid for the population, Σ = population variance which explains data dispersion around the centroid (Shah and Gemperline, 1989). Usually, a $D^2>3.0$ is considered significant separation between two E-nose data set clusters (Mark and Tunnell, 1985).

2.5.3 Application of the electronic nose technology

The E-nose has been successfully used in a wide range of fields for product matching and quality control of raw materials through processing and storage lines (Madsen and Grypa, 2000). E-noses have been used to monitor by volatiles emissions the ripeness of bananas, apples, mangoes, avocadoes (Giese, 2000), melons (Benady *et al.*, 1995), black truffles (Persaud and Talou, 1996) and mushrooms (Keshri *et al.*, 2003). Different types of spices (Madsen and Grypa, 2000), varieties of coffee (Aishima, 1999), detection of microbes and heavy metals (Canhoto and Magan, 2003), smells of

liquor and perfume, and quality of pharmaceutical products were also determined using E-noses (Coghlan, 1994). In addition, volatiles production by micro-organisms and insects enable the use of E-nose to detect spoilage fungi (Keshri and Magan, 2000), spoilage bacteria and yeasts in milk (Magan *et al.*, 2001; Korel and Balaban, 2002), and storage qualities of tomato (Sinesio *et al.*, 2000), meat, fish, edible oils and fats (Adechy *et al.*, 2000). However, there is no report in the literature on the use of E-nose for onion evaluation.

A newer volatile sensing technology is the recent introduction of an electronic tongue (zNose), a GC-like device with a SAW (quartz crystal) sensor, which is even faster (i.e. 10 s response time) and can measure the concentration of individual chemicals within volatiles compared to the conventional E-nose, but expensive and destructive (Staples, 2000).

2.5.4 Electronic nose sensor types and factors affecting conducting polymer sensor response to odour molecules

Table 2. Examples of commercial and laboratory sensor types for E-noses (after Gardner and Bartlett, 1999; Payne, 1998; Ziegler *et al.*, 1998).

Active material	Sensing principle	Manufacturers
Conducting polymers	Conductance	Osmetech, UK
Metal oxide e.g. SnO_2	Conductance	Alpha MOS, France
MOSFETs	Conductance	Nordic Sensor, Sweden
Infra-red CO_2 detectors	Conductance	Nordic Sensor, Sweden
Si/Pt pellistors	Pellistors	TU Munchen, ITE, Germany
QCM	Oscillation frequency	Lennartz electronic, Germany
SAW resonator	Oscillation frequency	RST Rostock, Germany
BAW resonator	Resonant frequency	RST Rostock, Germany
Electrolyte cells	Microelectrode array	Institute of Microtechnology, Switzerland
Electrolyte cells	Amperometric	Institute for Chemo- and Biosensorics, Germany

MOS, metal oxide sensor conductors; QCM, quartz crystal microbalances; MOSFET, metal oxide semiconductors field effect transistors; SAW, surface acoustic waves; BAW, bulk acoustic waves.

E-nose technology is based on gas sensors, which can be classified according to the type of materials used to build individual sensor elements (Gardner and Bartlett, 1999). The various sensor elements differ in their sensing principle and also their interaction with the chemical properties of presented odour molecules (Table 2).

Sensor materials produces signature of the volatile molecules, which register as odour 'fingerprints' (Ziegler *et al.*, 1998). Multiple sensor E-noses produce complex multiple odour profile patterns. These multiple E-nose sensor systems can enhance resolution of differences between similar samples as compared with a single sensor E-nose. An example is the 32-conducting polymer sensor E-nose (Fig. 6). Each of the multiple sensor elements has a unique sensing property that helps maximise sensitivity to particular chemical groups as each element measures a different chemical property of the sensed volatile compound (Giese, 1999; Osmetech, 2001). Conducting polymer sensors are readily available and easy to manufacture (Bartlett *et al.*, 1997).

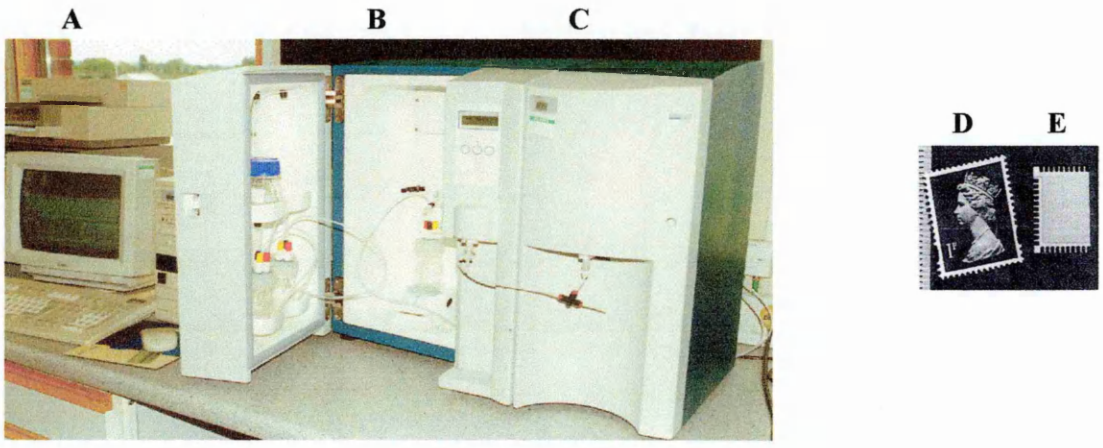

Figure 6. A 32-conducting polymer sensor E-nose (A32/8S; AromaScan (Osmetech), UK). A, Computer processor unit; B, E-nose sample unit; C, E-nose sensor unit and D, 1 penny UK stamp compared to E, the size of a 32 conducting polymer sensor chip.

Sensor elements are derived from thiophene (C_4H_5S), pyrrole (C_4H_5N) and aniline (C_6H_7N) monomers. These polymers expand on adsorption of odour molecules, leading to modulation of inherent conduction characteristics to electrons, which changes the rate of charge flow. Ideally, the odour molecule is immediately desorbed after the interaction. This response is measured as a fractional change in resistance

with reference to a base resistance and is somewhat specific to a particular odour compound (Benady *et al.*, 1995; Osmetech, 2001).

The response of a conducting polymer sensor, like other E-nose sensors, is subject to sensor and sample drifts (K. Persaud, pers. comm., 2001). Sensor drifts are caused by irregularities in temperature and relative humidity and also by compounds forming protective layers on sensor elements, such as hexamethyldisiloxane (Bartlett *et al.*, 1997; Kohl, 1997). Sample drifts are caused by biochemical changes in the composition of headspace volatiles during sample preparation and handling (K. Persaud, pers. comm., 2001). These drifts can contribute to lack of repeatability between replicates of the same sample and can thereby affect interpretation of results. Conducting polymer sensors can be 'poisoned' or inhibited by carbon and/or sulphur-containing compounds. Chemisorption occurs when sulphur irreversibly binds to reactive sites on the sensor materials (Kohl, 1997; Gardner and Bartlett, 1999). Headspace equilibration to specified temperature and RH values and intermittent flushing and washing of sensors could help reduce sensor drifts (Bartlett *et al.*, 1997; Payne, 1998).

Thiosulphinate concentrations (Peaks 6 to 15 in Fig. 7) in homogenised onion sample generally do not change appreciably over and up to 8 h at room temperature (Block *et al.*, 1992). The only obvious changes in headspace volatile compounds were complete lost of LF in <30 min of homogenisation and a steady reduction in (E)-1-propenesulfinothioic acid S-methyl ester (peak 10), especially after 24 h. The S compounds and ammonia in onion homogenate caused difficulties when E-nose was first tested to analyse onion samples (B. Smith, pers. comm., 2000). This failure suggests that standardisation of sample preparation and handling is very important. Sampling for onions might be safe for up to 8 h in accordance with the work of Block *et al.* (1992) without affecting flavour composition, although LF will be absent.

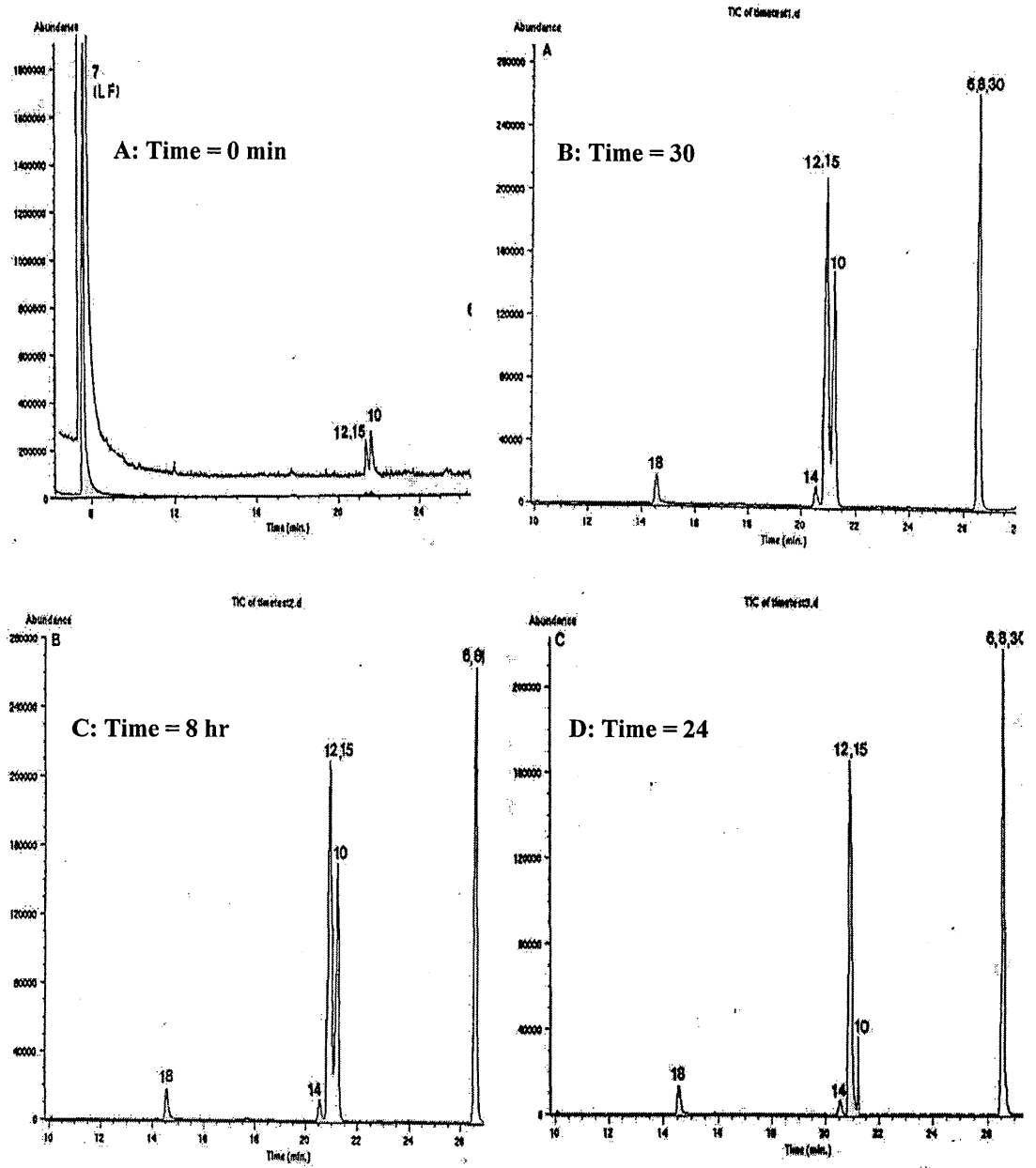

Figure 7. GC-MS chromatogram for white onion thiosulphinates as affected by time of homogenisation prior to extraction and subsequent analysis. Peak numbers are 6, 1-propanesulphinothioic acid S-(Z)-1-propenyl ester; 8, 1-propanesulphinothioic acid S-(E)-1-propenyl ester; 10, (E)-1-propenesulphinothioic acid S-methyl ester; 12, methanesulphinothioic acid S-(Z)-1-propenyl ester; 14, methanesulphinothioic acid S-1-propyl ester; 15, methanesulphinothioic acid S-(E)-1-propenyl ester; 18, methanesulphinothioic acid S-methyl ester; 30 and 31, zwiebelanes (cis and trans; after Block *et al.*, 1992).

2.5.5 Prospects and constraints of electronic nose technology

The E-nose has proved useful in many different fields including the food industry for sample analysis, quality control and product matching (Madsen and Grypa, 2000). Large numbers of samples can be analysed in a given time and at a lower cost as compared with sensory and analytical tests. Sensor life span and reliability have improved (Ulmer *et al.*, 1997). Sample replication in addition to sensor flushing and washing using AromaScan computer software (AromaScan service manual, 1998) during running of samples reduces sensor-poisoning effects to maximise reproducibility of E-nose data set (Payne, 1998). The robustness of the E-nose and development of portable hand-held models (e.g. Cyranose 320; Cyranose Sciences Inc., www.cyranosesciences.com) will enhance portability and ease of use (Payne, 1998, Giese, 2000). The recent developments of hybrid E-noses (e.g. Agilent 4440A MS-E-nose; Adechy *et al.*, 2000) can further improve response signal interpretation and understanding. This system combines E-nose and mass spectrophotometer and operates by detection of fragment ions ranging between 25 and >150 m/z in headspace volatiles. Several companies are developing and marketing improved versions of E-noses that are becoming less expensive to buy and use. These companies include Osemetech (Hollis, Northampton, UK), Cyrano Sciences (Pasadena, California, USA), AlphaMOS (Hillsborough, NJ, USA), and Nordic Sensor Technologies Inc. (Jersey City, NJ, USA) (see Table 2 for more manufacturers). Market promotion will create awareness and boost sales of these E-noses.

While the E-nose has potential, notable limitations are the current high price of the instrument and difficulty in interpretation and understanding output data response patterns (Payne, 1998; Sinesio *et al.*, 2000). Currently, E-nose patents are own by few firms that gain higher profits in the health care sector compared to the food industry. For example, 52 patents for conducting polymers and microbiological E-noses are owned by Osmetech (Osmetech, 2001) and Zakacs and Bodall (2002) at the University of West Yorkshire owned two patents for conducting organic polymers E-nose. In the US sensor arrays for detection of microorganisms assigned to California Institute of Technology, odour sensor assigned to Bloodhound Sensors, method for conservatively evaluating continuous thermal treatment process for a particulate-

containing food product stream assigned to North Carolina State University and reflection measuring device and method for determination of quality properties of items assigned to Slagteriernes Forskningsinstitut (Giese, 2000).

The E-nose assesses total composite headspace gases that may include flavour and non-flavour compounds. Consequently, odour fingerprints do not necessarily correlate with actual smell and taste perception (Madsen and Grypa, 2000). E-noses with sensors that do not react to fluctuations in temperature and relative humidity are yet to be developed (Payne, 1998). In spite of precautions taken to ensure sensor consistency, differences between replicates of the same sample are experienced, which cannot yet be explained (Bartlett *et al.*, 1997).

2.6 Conclusion

Growth and quality of onions and other Alliums vary widely among genotypes and species grown under different environment conditions and management practices. For instance, temperature, soil type, irrigation and N:S ratio affect flavour intensity and storage quality. Further work is required to more fully document and better understand interaction effects. Processing and packaging techniques, postharvest environment conditions and storage duration all markedly influence the shelf life, of both intact and more so minimally processed onions. Conventional sensory and analytical determinations of onion flavour are constrained by high cost, complexity and time-consuming issues. Thus, demonstration of a successful application of relatively new E-nose technology would be advantageous in the contexts of both experimental and commercial onion quality monitoring. The present study investigates the potential for use of the E-nose in discrimination of differences in headspace volatiles for Alliums. Treatment variables include genotype, N, S, soil type and irrigation regime. Effects of these treatment variables on growth, yield and quality are also recorded. Storage quality of minimally processed onion wrapped in polyethylene bags was also monitored using the E-nose.

CHAPTER 3: *ALLIUM* TYPES EXPERIMENT

3.1 Discrimination amongst *Alliums* using an electronic nose

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(*Annals of Applied Biology* **139**, 337-342)

Summary

Pyruvic acid content determination and, to lesser extents, thiosulphinates determination and organoleptic tests are used for assessing the eating characteristics of onions and other *Allium* spp. Each of these methods has inherent limitations, especially when large numbers of samples are to be evaluated. With a view to developing a more convenient quality evaluation method, an electronic nose was used to discriminate flavour and aroma characteristics amongst garlic, leek, shallot, bulb onion and spring onion. Differences in relative sensor response to headspace volatiles over macerated samples of these five different *Allium* types were recorded. Principal Component Analysis (PCA) showed some separation among the five types. PCA and Mahalanobis' D^2 statistic suggested similarities in headspace volatiles for shallot, spring and bulb onions and differences for leek and, especially, garlic. Multiple linear regression analyses ($Y = \alpha + \beta_1 X_1 + \beta_1 X_2$; $N_{(X, Y)} = 5$) of the first two principal component values (PCA 1 [X_1] and PCA 2 [X_2]) accounted for not less than 90% of the total variation in pyruvic acid concentration (Y1), total soluble solids content (Y2) and percentage dry matter content (Y3) of the *Allium* types. These relationships suggest that electronic nose discrimination was on the basis of quality characteristics that relate to *Allium* flavour. This work has shown that the electronic nose has potential for flavour characteristic-based discrimination amongst *Allium* types. Future work will explore this potential within a single *Allium* spp.

Key words: *Allium* species, dry matter, electronic nose, headspace, onion, pyruvic acid, total soluble solids

Introduction

Allium spp. are valued for their characteristic taste and aromas. Flavours of *Allium* spp. are largely due to volatile organosulphur compounds released from precursors collectively referred to as S-alk(en)yl-L-cysteine sulphoxides (ACSOs). These compounds are acted upon by the enzyme alliinase during tissue disruption (Block, 1992; Block *et al.*, 1992; Randle, 1997). *Allium* spp. contain four ACSOs; namely, (a) S-1-propenyl-, (b) S-2-propenyl-, (c) S-methyl-, and, (d) S-propyl-L-cysteine sulphoxides (Block, 1992). Differences in composition and amount of ACSOs in *Allium* spp. have been reported. For instance, Block (1992) reported that varying amounts of compounds 'a', 'c' and 'd' are found in onions, while 'b', 'c', and 'd' are found in garlic.

Allium spp. have been classified chemotaxonomically with respect to the nature and relative abundance of thiosulphinates and related compounds (Block *et al.*, 1992; Thomas & Parkin, 1994). Sensory appraisals of onion flavour and pungency correlate positively with concentrations of certain non-protein sulphur amino acid compounds, such as the thiosulphinates, thiopropanal sulphoxide (the lachrymatory factor), and pyruvic acid (Freeman & Whenham, 1975). These compounds are produced during tissue disruption and subsequent hydrolysis of ACSOs by alliinase (Lancaster & Kelly, 1983; Block, 1992; Randle, 1997). Hydrolysis of ACSOs yields ammonium pyruvate and an unstable sulphenic acid, which rapidly decomposes to thiopropanal sulphoxide (Freeman & McBreen, 1975; Block, 1992; Thomas *et al.*, 1992). Although the detailed chemistry of this reaction has been debated (Thomas *et al.*, 1992; Thomas & Parkin, 1994), pyruvic acid determination is relatively simple and is used successfully to measure *Allium* flavour (Randle, 1992; Ashish *et al.*, 1995; Debaene *et al.*, 1999).

The electronic nose is a relatively novel device used for volatiles sensing. Headspace volatiles are sorbed by an array of tiny sensors. Electronic nose sensors are classified, on the basis of construction material used, as inorganic crystalline or polycrystalline, organic or polymers, and biologically derived (Gardner & Bartlett, 1999). Sensors made from organic materials, such as conducting polymer sensors, are typically constructed from eight to 48 different polymers arranged as networked individual units. Polymers include pyrrole, aniline and thiophene monomers. Their resistance to an electric current reversibly changes in response to absorption of aroma

molecules (Gopel, Ziegler *et al.*, 1998; Gardner & Bartlett, 1999). A complex electronic signal is generated by the sensor array and analysed by computer (Gopel *et al.*, 1998; Gardner & Bartlett, 1999). Electronic nose technology has been successfully used to discriminate quality and flavour of various products, including tomatoes (Sinesio *et al.*, 2000), spices (Madsen & Grypa, 2000) and black truffles (Persaud & Talou, 1996). The technology may also be used to assess quality of stored grain, fish, drugs and drinks (Bartlett, Elliot & Gardner, 1997) and food spoilage (Adechy *et al.*, 2000).

Difficulties in discriminating between onion samples using an electronic nose have been encountered in early unpublished work. This difficulty was attributed to the sulphur-containing flavour compounds and to ammonia production in disrupted tissue (B Smith, personal communication). Sulphur-containing compounds can have a poisoning or inhibitory effect, referred to as chemisorption, by irreversibly binding to reactive sites in sensor materials (Kohl, 1997; Gardner & Bartlett, 1999). Conducting-polymer sensors, such as those based on polypyrrole, polythiophene and polyaniline, may enhance the reversibility of sulphur compound chemisorption (A Tummon, personal communication). Conducting polymer sensors undergo rapid adsorption and desorption kinetics and, therefore, are resilient to poisoning on interaction with, for example, sulphur-containing compounds (Kohl, 1997).

This study investigated the use of an electronic nose (A32/8S AromaScan) based on a conducting-polymer for discrimination between garlic (*A. sativum* L.), bulb and spring onions (*A. cepa* L.), shallot (*A. ascalonicum* auct. non Strand) and leek (*A. ampeloprasum* L.).

Materials and Methods

Procurement and preparation of samples

Various *Allium* spp. were obtained from a local supermarket. Edible parts of garlic (clove), shallot (bulb), leek (pseudostem), onion (bulb) and spring onion (13 to 15 cm long portions measured from the stem-plate end up to the leaf blade) were homogenised in a Moulinex (TIPO 753; PATENDO, Spain) mixer at room temperature. The slurry was filtered after 20 min, and 20 ml of the filtrate was transferred to a 250 ml conical flask. Twenty ml of 50 g trichloroacetic acid (TCA) in

1 litre deionised water was added to terminate alliinase activity. The mixture was agitated vigorously and allowed to stand for 1 h.

Electronic nose evaluation

Deionised water (10 ml) was added to 10 ml of filtrate/TCA solution and mixed. One ml of this diluted solution was put into a 100 ml Schott bottle and placed in the sample station of an AromaScan LabStation System A32/8S (Osmetech, UK) to equilibrate for 10 min at 25°C and 30% r.h. Headspace gas was sampled for a period of 70 s at a gas flow rate of 50 ml min^{-1}. Percentage change in resistance ratio with reference to a base resistance (%dR/R) response of the electronic nose 32 conducting polymer sensor unit to headspace volatiles was recorded. %dR/R is calculated from [Ro - Rs / Ro * 100]; where Ro is base resistance and Rs is measured resistance due to sensor interaction with odour molecules.

Pyruvic acid determination

Total pyruvic acid concentration was determined using fresh samples. For background pyruvic acid concentration, samples of the different *Allium* types were boiled for 25 min, frozen and then thawed after 24 h. The difference between total and background pyruvic acid concentration (i.e. enzymically produced pyruvic acid) was the measure of alliinase activity. One ml each of 125 mg 2,4-dinitrophenylhydrazine in 1 litre of 2 M HCl solution and deionised water were added to a 1 ml aliquot of the filtrate/TCA solution prepared as described above (see procurement and preparation of sample) and mixed. This mixture was heated in a water bath at 37°C for 10 min, after which 5 ml of 0.6 M NaOH was added and mixed. Standard solutions of 0 to 0.3 mM pyruvic acid were prepared with sodium pyruvate following the procedure above. A UV/VIS spectrophotometer (Model PU8730; UNICAM, UK) was used to determine the absorbance of samples and standards at 420 nm (Schwimmer & Weston, 1961; Randle & Bussard, 1999).

Dry matter and total soluble solids contents

Edible portions (100 g) of each species were oven-dried at 60°C to constant weight and dry matter content was calculated on a fresh weight basis. Total soluble solids (TSS) content was measured using a digital refractometer (Model PR1; Atago Co.

Ltd., Japan) after diluting the slurry (without TCA) of each *Allium* type with an equal volume of deionised water.

Experiment design and data analysis

A completely randomised design with four (five for electronic nose evaluation) replications was adopted. Responses of the 32 conducting polymers sensor were analysed and processed using A32S Microsoft Windows Version 3.24B software (AromaScan Plc., UK). A two-dimensional PCA plot was obtained. Each principal component (PCA 1 and PCA 2) explains percentage variance in the data (Wold *et al.*, 1987; Gardner & Bartlett, 1999). Eigenvalue (variance) was calculated by dividing the fraction of explained variance by the number of variables (Wold *et al.*, 1987). Separations between centres of clusters on the PCA plot were determined by Mahalanobis distance (D^2) with the A32S Microsoft Windows software. The Mahalanobis D^2 statistic is a measure of multivariate distance that is calculated base on distribution of data sets, cluster size and cluster orientation (Gardner & Bartlett, 1999). D^2 statistic measures the magnitude of divergence between two clusters (Mahalanobis, 1941). Location of clusters was determined by using the minimum D^2 classification rule, which indicates that an observation 'x' is assigned to a population 'i' if $Di^2 < Dj^2$; 'i' $\neq$ 'j' (Morrison, 1976; Gnanadesikan, 1977; Mark & Tunnell, 1985). $D^2 < 3$ (i.e. three standard deviations) between two groups was considered as similar (Mark & Tunnell, 1985). One-way analyses of variance (ANOVA) were carried out using Minitab for Windows Version 12.13 (Minitab Inc., USA) software. Percentage change in resistance of the 32 electronic nose sensors was averaged and transformed using the method of square root transformation i.e. $[\%dR/R + 0.5]^{0.5}$ before ANOVA, since all data were less than 30% (Gomez & Gomez, 1984). Treatment means were separated by the least significant difference (LSD) test at the 5% significance level. Multiple linear regression equations using Minitab for Windows software were used to account for variations in each of total pyruvic acid concentration, total soluble solids and percentage dry matter contents with PCA 1 and PCA 2 as the predictors (Gomez & Gomez, 1994).

Results and Discussion

There were differences ($P < 0.01$) in response (%dR/R) of the electronic nose sensor for the five *Allium* types (Table 1). Data from the 32 conducting polymer sensor are

Table 1. *Electronic nose sensor response (%dR/R) to headspace volatiles (N = 5), pyruvic acid concentrations (N = 4), total soluble solids (N = 4) and dry matter (N = 4) contents for five* Allium *types*

			Pyruvic acid concentration			Total soluble solids	Dry matter
	[a][%dR/R + 0.5]$^{0.5}$		(µM g-1 fresh weight)			(%)	(%)
Allium types	Expt 1	Expt 2	Total	Background	Enzyme		
Onion	1.6	1.6	13.9	5.1	8.7	4.4	10.2
Shallot	1.8	1.9	12.5	5.4	7.2	5.8	13.2
Spring onion	1.7	1.9	6.6	5.6	1.0	2.6	7.9
Leek	2.3	2.5	9.2	5.3	4.0	4.6	11.7
Garlic	2.5	2.5	29.6	10.4	19.2	18.0	36.7
LSD (5%)	0.15	0.25	0.51	0.44	-	0.71	1.65
CV (%)	5.7	8.7	2.0	3.8	-	5.5	5.7

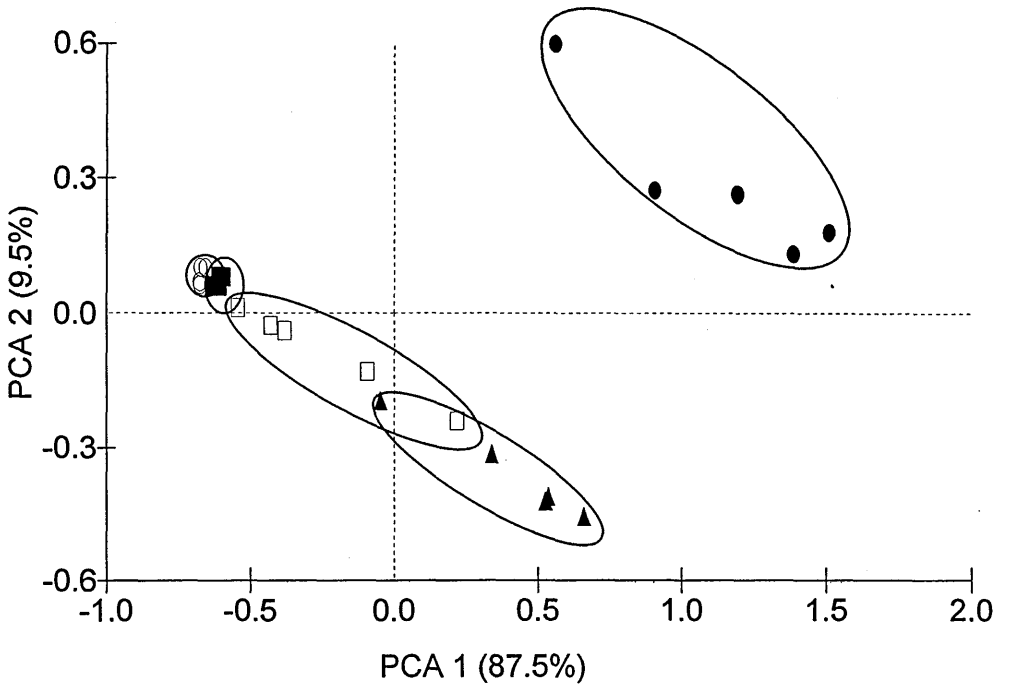
[a]Square root transformation of %dR/R

mapped in Fig. 1 by two-dimensional Principal Component Analysis (PCA) for each of duplicate experiments. Relative separation between *Allium* types on the PCA maps is reflected in the Mahalanobis distance (D^2) (Mahalanobis, 1941; Morrison, 1976; Gnanadesikan, 1977; Mark & Tunnell, 1985; Gardner & Bartlett, 1999) values presented in Table 2. Garlic was distinctly different from the other four *Allium* types

Table 2. *Mahalanobis distance (D^2) indicating separation distance between 2D PCA plot clusters (Fig. 1) for headspace volatiles of edible portions for five different* Allium *types (N = 5)*

	Garlic	Leek	Onion	Shallot
PART A – Expt 1				
Leek	3.6	-	-	-
Onion	5.8	2.6	-	-
Shallot	11.6	5.9	1.4	-
Spring onion	13.6	8.0	3.4	3.6
PART B – Expt 2 (repeat)				
Leek	3.8	-	-	-
Onion	4.4	2.2	-	-
Shallot	15.0	10.3	3.7	-
Spring onion	13.7	9.7	3.7	1.2

A (Experiment 1)



B (Experiment 2; repeat)

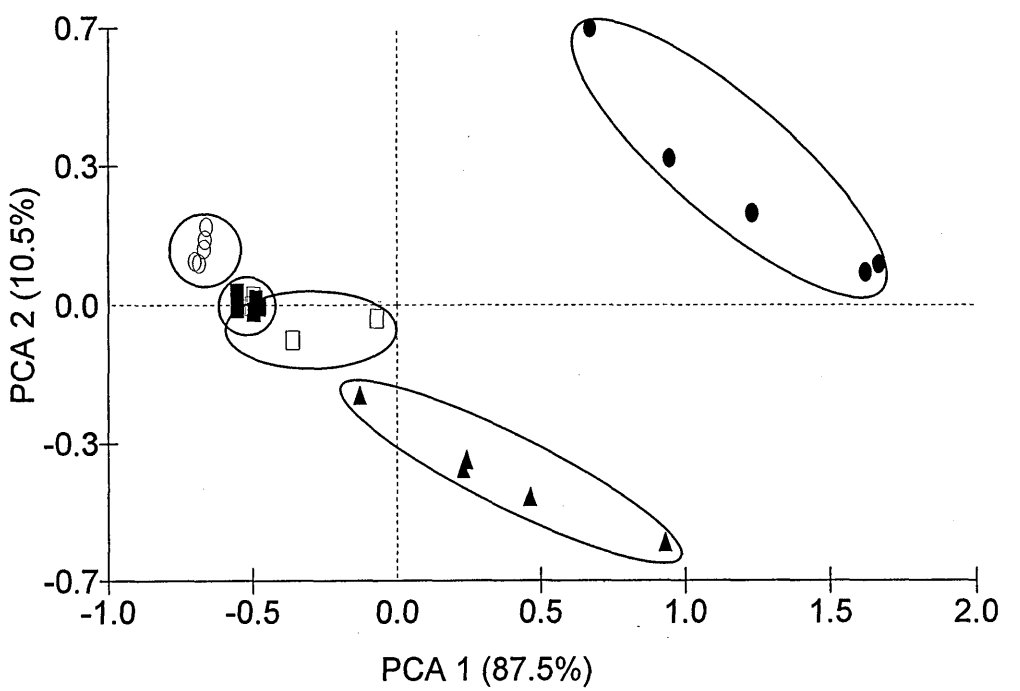


Fig. 1. 2D Principle Component Analysis (PCA) maps for repeated electronic nose experiments (A, B) using headspace volatiles of onion (□), shallot (■), spring onion (○), leek (▲) and garlic (●).

(Fig. 1, Table 2). Differences may be ascribed to the nature and abundance of thiosulphinates in the five different *Allium* types. Given inherent differences in thiosulphinate compound characteristics amongst these *Allium* types, the data suggest that the polymer sensors respond differentially to various dominating chemical groups in the headspace. The 2D PCA map (Fig. 1) obtained from principal components PCA 1 and PCA 2 explained over 97% of the total variance in sensor response to headspace volatiles of the five *Allium* types. On average, PCA 1 alone accounted for over 87% (mean eigenvalue = 4.4) of the total variance, while PCA 2 accounted for 9% (mean eigenvalue = 0.5). This finding suggests that headspace volatiles of *Allium* types may be characterised by the first principal component (PCA 1) without significant loss of information (Wold *et al.*, 1987).

The amount of pyruvic acid produced due to alliinase activity following tissue damage (homogenisation) was 15% in spring onion (Table 1). In the other *Allium* types, enzymically-produced pyruvic acid was much higher and ranged between 43 and 65%. These differences in alliinase activity may be explained by morphological differences amongst the edible portions sampled. Spring onion is comprised of a large proportion of green leaf tissue (see materials and methods). The biochemistry of leaf tissue is generally different from that of the swollen leaf bases of bulb onions and shallots, the cloves of garlic and the pseudostem of leek (Brewster, 1994). Lancaster, Shaw & Walton (2000) reported low concentrations of flavour precursors and alliinase activity in the leaves of leucocoryne (*Leucocoryne spp.*), as compared to bulbs and scapes. This observation supports the data showing a low concentration of enzymically-produced pyruvic acid in spring onion. Garlic had the highest background pyruvic acid content, while only slight differences were recorded amongst onion, shallot, leek and spring onion. Values of total, background and enzymically-produced pyruvic acid concentrations for the five *Allium* types generally agree with previous reports (Freeman & Mossadeghi, 1970; Freeman & Whenham, 1975; Ashish *et al.*, 1995).

Percentage dry matter (%DM) and total soluble solids (TSS) contents of garlic were higher ($P < 0.01$) than those of the other four types (Table 1). The lowest %DM and TSS contents were found in spring onion. Linear correlation analysis ($Y = \alpha + \beta X$; $N_{(X, Y)} = 5$) indicated a highly significant ($P < 0.01$) association between %DM and TSS content ($r = 1.00$). Both parameters had significant ($P < 0.05$) positive linear

relationships ($r > 0.90$) with each of total, background and enzymically-produced pyruvic acid. As noted earlier, pyruvic acid concentration in *Allium* spp. is a generally useful measure of pungency (Schwimmer & Weston, 1961; Thomas *et al.*, 1992; Randle & Bussard, 1999). Thus, these results confirm previous reports that *Allium* spp. with high dry matter and TSS also have high pungency.

Multiple linear regression (MLR) analyses ($Y = \alpha + \beta_1X_1 + \beta_1X_2$; $N_{(X, Y)} = 5$) indicated that the combination of PCA 1 and PCA 2 significantly ($P < 0.05$) accounted for variations in each of pyruvic acid concentration, %DM and TSS content of the *Allium* types (Table 3). The coefficient of determination for the MLRs suggested that PCA 1 and PCA 2 accounted for 90% of the total variation in pyruvic acid concentration, 97% of the total variation in %DM and 96% of the total variation in TSS content of the *Allium* types. These findings further suggest that the electronic nose data set for *Allium* types can be used to predict flavour as otherwise measured by pyruvic acid concentration, percentage dry matter and total soluble solids contents.

Table 3. Multiple linear regression ($Y = \alpha + \beta_1X_1 + \beta_1X_2$; $N_{(X, Y)} = 5$) of pyruvic acid concentration, total soluble solids and percentage dry matter contents on principal components one (PCA 1) and two (PCA) obtained from pooled mean data sets for five different Allium *types evaluated using electronic nose*

Physiological variate (Y)	Model	R^2	Significance	SE
Pyruvic acid				
($\mu M\ g^{-1}$ fresh weight)	$Y = 14.4^{**} + 8.5^{*}$ PCA 1 + 18.2^{ns} PCA 2	90%	$P = 0.10$	1.7
Total soluble solids (%)	$Y = 7.1^{***} + 6.3^{**}$ PCA 1 + 12.0^{*} PCA 2	96%	$P = 0.05$	0.7
Dry matter (%)	$Y = 16.0^{**} + 12.1^{*}$ PCA 1 + 22.5^{**} PCA 2	97%	$P = 0.05$	1.2

$^{*}, ^{**}, ^{***}$Significance at the 10%, 5% and 1% levels, respectively; ^{ns}not significant; SE, standard error of the multiple linear regression

This investigation shows that the electronic nose can discriminate between some *Allium* types with respect to their headspace volatiles and pungency. It also confirms previously established positive correlations between pyruvic acid content (pungency), dry matter and TSS level in *Allium* spp. Ongoing work will determine whether the electronic nose has the potential to discriminate qualitative characteristics among onions grown on different soils under different nutrient (nitrogen and sulphur) and irrigation regimes.

Acknowledgement

The authors thank Dr Brian Smith and Mr Andrew Tummon of Horticultural Research International and OSMETECH, respectively, for their advice concerning the electronic nose. We are also grateful to Mr Charles Marshall for his advice on statistics.

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CHAPTER 4: SPRING ONION EXPERIMENTS

4.1 Genotype, sulphur nutrition and soil type effects on growth and dry- matter production of spring onion

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(*Journal of Horticultural Science and Biotechnology* 77(3), 340-345)

SUMMARY

Effects of genotype, sulphur nutrition (0.0, 2.9, 5.8 kg S ha^{-1}) and soil type (clay, sandy loam) on spring onion growth were investigated in glasshouse experiments. Leaf greenness, number of green leaves, leaf length, bulb diameter, total plant fresh weight, percentage dry-matter (%DM) and total soluble solids content (TSS) varied significantly ($P<0.05$) among eight spring onion genotypes grown on sandy loam. Overall, *A. cepa* cultivars had more green leaves, taller plants and greater bulb diameter both with (5.8 kg S ha^{-1}) and without S application than *A. fistulosum* cultivars. Deficiency symptoms of chlorosis, retarded growth and tip-burn were observed for plants not fertilised with S. S application differentially influenced TSS among genotypes. For example, S fertilisation did not affect TSS of 'Paris Silverskin', but TSS was reduced by 30% in 'Sydney Bunching'. Increased TSS was correlated with increasing %DM ($r = 0.77$). On average, *A. cepa* cultivars were more responsive to applied S than *A. fistulosum* cultivars. More efficient S utilisation resulted in greater increases in plant growth, bulb diameter and DM yield. For 'White Lisbon', increased S application from 0.0 to 2.9 kg ha^{-1} increased growth. There was no further increase when S was applied at 5.8 kg ha^{-1}. TSS was reduced in response to added S. Growth of 'White Lisbon' was greater on clay than on sandy loam. However, TSS was not affected by soil type.

Spring onion (*Allium* spp.) is a member of the 'Common' onion group (Hanelt, 1990). The whole plant can be eaten as green or salad onion (Rubatzky and Yamaguchi, 1997). For late summer production, *A. fistulosum* cultivars are substituted for *A. cepa* because of the latter's reduced tendency to form bulbs under longer days (Brian Smith, pers. comm.). Anecdotal evidence from growers suggests that *A. fistulosum* cultivars generally have a more erect foliage habit, poorer texture and stronger flavour than *A. cepa* cultivars (Brian Smith, pers. comm.).

Growth, yield and flavour intensity of spring and bulb onions are dependent on genotypic characteristics modified by climatic conditions, edaphic factors and management practices (Freeman and Mossadeghi, 1970; Randle, 1992a; 1992b; Randle *et al.*, 1994; Hamilton *et al.*, 1997; Abbey and Fordham, 1998). Sulphur (S) nutrition is important in determining yield and quality of many crops, including onions (Freeman and Mossadeghi, 1970; Randle, 1992a; 1992b; Hamilton *et al.*, 1997; 1998; Hartmann *et al.*, 2000; Hawkesford, 2000). S deficiency during growth causes leaf chlorosis and necrosis, especially of the youngest leaf, and stunts plant growth (Freeman and Mossadeghi, 1970; Salisbury and Ross, 1992; Hawkesford, 2000).

Atmospheric S in rainwater is a major supply of S to plants (MAFF, 1994). Since 1970, there has been 40% reduction in atmospheric input of S to soils in the UK. This reduction may adversely affect production of S sensitive crops, such as onions. Nutrient imbalances (especially in the N:S ratio), soil temperature extremes, water-stress and soil type all affect populations and activities of micro-organisms involved in S oxidation prior to S uptake by roots (Jones, 1986; Konopka *et al.*, 1986; Stroehlein and Pennington, 1986; Hawkesford, 2000). S assimilation and utilisation efficiency are thereby affected (Salisbury and Ross, 1992; Lambers *et al.*, 1998). Freeman and Mossadeghi (1970) found that growth of 'White Lisbon' at 0.1 meq (3.2 mg) S l^{-1} was reduced by 50% compared with 3.0 meq (96.2 mg) S l^{-1}.

Most studies on growth and quality of *Allium* spp. have focused on bulb onions. The National Institute of Agricultural Botany (NIAB) conducts spring onion trials every year to select good cultivars for UK growers (NIAB, 2000). However, little is known about the effect of environment and management factors on spring onion growth and on the yield performance of various cultivars.

This study evaluates the responses of eight spring onion cultivars to varied S nutrition, and determines the interactive effects of soil type by S nutrition on growth

and dry-matter production of one of these spring onion cultivars. Relationships between dry-matter content and total soluble solids (TSS) content are reported.

MATERIALS AND METHODS

Two glasshouse experiments were carried out on spring onions between April and December 2000.

Plant material

Seeds of seven *Allium* cultivars, consisting of two species of spring onion, were obtained from the Genetic Resources Unit, Horticultural Research International (HRI). Four cultivars of *A. cepa*: Winter White Bunching, Egyptian Bunching, Winter Over and Paris Silverskin; two of *A. fistulosum*: Fragrant and Sydney Bunching; and Guardsman, a hybrid from *A. cepa* x *A. fistulosum* cross were used. Also, seeds of *A. cepa* 'White Lisbon', an "all-year-round" cultivar, were purchased from a seed supplier (E. W. King and Co. Ltd., Monks Farm, Colchester). Seeds were pre-germinated at 25°C in 8.5 cm diameter Petri dishes lined with moistened filter paper. Five seedlings were transplanted into each pot 5 d after germination. Seedlings were irrigated with distilled water.

Soil preparation and pot filling

The soils used were clay (Alluvial Gley soil; Thames series, T_s) and sandy loam (Brown Earths; Wick series, WQ_2) (Whitfield, 1974). Soil types were chosen as being representative of soils in onion growing regions worldwide (Lorenz and Maynard, 1988). The clay soil was dug from a fallowed field and the sandy loam from a continuously cropped field at the HRI Experimental Farms, Wellesbourne. The soils were air-dried in a glasshouse to moisture contents of 9.9% (clay) and 2.6% (sandy loam) on an oven-dry weight basis. The air-dried soils were passed through a 2 cm^2 mesh sieve. Water-soluble sulphate (1:5 soil/water extract) and nitrogen (Kjeldhal method) contents of the air-dry soils (Table I) were determined.

Plastic pots of 12 cm diameter placed in saucers were used in the experiments. These were bottom-filled with 100 g washed grit to improve aeration and drainage. Either clay (500 g air-dried weight) or sandy loam (700 g air-dried weight) was added and compacted to fill approximately 500 cm^3 of the pot.

TABLE I

Physical and chemical characteristics of the clay and the sandy loam soils used in experiments with spring onions

Parameter	Clay	Sandy loam
Soil series (WQ_2)	Thames (T_s)	Brown Earths
Particle density (g cm^3)	2.1	2.5
Dry bulk density (g cm^3)	1.1	1.4
Water-soluble sulphate (mg kg^{-1})	62.6	40.6
N (g kg^{-1})	68.7	31.6
Water content (ml kg^{-1} air-dried soil)		
Field capacity (-0.01 MPa SWP[1]); A	393.0	149.0
Permanent wilting point (-0.16 MPa SWP); B	281.0	64.0
Available water-holding capacity (A-B)	112.0	83.0

[1]SWP, soil water potential.

Nutrient supply

Nutrient solution was applied in split applications of half the recommended rate, two and five weeks after transplanting. The mineral nutrients applied per pot were 120 mg N (urea, $CO(NH_2)_2$), 24 mg P (metaphosphoric acid, H_3PO_4), 72 mg K (muriate of potash, KCl) and 24 mg Mg (magnesia, MgO). S was applied in the form of epsom salt (magnesium sulphate, $MgSO_4$) based on MAFF (1994) recommendations.

Genotype and S nutrition experiment

Seeds of eight spring onion cultivars, Winter White Bunching, Egyptian Bunching, Winter Over, Paris Silverskin, White Lisbon, Fragrant, Sydney Bunching and Guardsman were pre-germinated on 9 June 2000. Five seedlings were transplanted into each pot containing sandy loam soil after 14 June 2000. Plants were supplied with or without 5.8 kg S ha^{-1} (246.2 mg hydrated $MgSO_4$ per pot) in split applications of half the total amount at two and five weeks after transplanting. A randomised complete-block factorial design with five replications was used. Each block contained two pots of ten plants (five plants per pot) for each of ±S x eight cultivars. Final harvest was on 15 August 2000.

S nutrition and soil type experiment

Seeds of cv. White Lisbon were pre-germinated on 6 August 2000 and transplanted after 5 d. Plants were grown on either clay or sandy loam soils. Plants received 0.0, 2.9 or 5.8 kg S ha^{-1} in two splits as described above. The experiment design was a randomized complete-block factorial (soil type x S nutrition level) with three replications. Each treatment combination per replication comprised 16 pots of 80 plants (five plants per pot). Final harvest was on 28 November 2000.

Data collection

Visual observations were made of leaf colour and plant growth until 10 and 16 weeks after transplanting (WAT), which were the final harvest times for genotype versus S and S versus soil type experiments, respectively. Total plant fresh and dry weights for the S versus soil type experiment were determined from four pots each containing five plants at 17, 52, 64 and 98 d after transplanting. At harvest, leaf greenness of the youngest, approximately 5 mm diameter, leaf was measured by SPAD value using a Minolta chlorophyll meter (SPAD-501; Minolta Camera Co. Ltd., Japan). The number of green leaves per plant, including newly emerged ≥1 cm long leaves, was also recorded. The lengths of leaf and plant height were measured from the tip of the longest leaf down to the point of attachment to the pseudostem and the base of the bulb (stem-plate) excluding roots, respectively. Bulb diameter was measured using a pair of callipers at 10 WAT at the broadest section of the bulb. Edible portions (pseudostem plus equal length of green leaves), remaining parts of the foliage and roots were weighed separately immediately after plants were harvested. These tissue samples were dried in an oven at 70°C to constant weight and their dry weights recorded. TSS was determined on homogenized edible portion using an Atago digital refractometer (Model PR1; Atago Co. Ltd., Japan).

Statistical analyses

Minitab for Windows Version 12.23 (Minitab Inc., USA) was used for analysis of variance (ANOVA) for the balanced designs and for fitting linear correlations. Data for numbers of green leaves were log transformed before ANOVA (Gomez and Gomez, 1984). The least significance difference (LSD) method was used to compare individual treatment means at $P = 0.05$ (Gomez and Gomez, 1984). SigmaPlot Version 5.0 (SPSS Inc., USA) was used to plot graphs and trend lines.

RESULTS AND DISCUSSION

Genotype and S nutrition

S deficiency symptoms of chlorosis with tip-burn, especially in the youngest leaves, and stunted growth were observed in the present study. These symptoms were not evident on S-fertilized plants. Previous workers have described similar symptoms among others of S deficiency for spring (Freeman and Mossadeghi, 1970) and bulb onions (Hartmann *et al.*, 2000), and also for other plant species such as spinach (*Spinacea oleracea* L.) (Warrilow and Hawkesford, 1998).

In the absence of added S (0.0 kg S ha^{-1}), SPAD values for leaf greenness were reduced (Table II). Upon application of S, the relative increase in leaf greenness varied among the eight spring onion genotypes. When S was not supplied 'Fragrant'

TABLE II

Leaf greenness, number of green leaves and leaf length at harvest after growing for ten weeks of eight spring onion cultivars as affected by genotype and sulphur nutrition

	Leaf greenness (SPAD value)		No. of green leaves per plant		Leaf length (cm)	
Cultivar	-S	+S	-S	+S	-S	+S
Winter Over	22.0	41.9	2.8	3.7	21.7	26.5
Paris Silverskin	37.4	43.6	3.2	4.3	24.0	25.7
White Lisbon	26.6	46.4	3.3	4.1	23.2	28.8
Sydney Bunching	19.0	36.8	4.3	5.0	23.9	26.5
Guardsman	19.4	39.4	3.4	4.0	24.0	27.6
Fragrant	17.2	36.2	4.4	4.7	21.2	24.6
Winter White	18.8	44.8	3.5	4.1	21.9	27.1
Egyptian Bunching	26.0	39.4	3.5	4.3	24.2	29.1
Mean	23.3	41.0	3.6	4.3	23.0	27.0
$LSD_{0.05}$						
Genotype (G)	7.83**		0.06**		2.26*	
Sulphur (S)	3.92**		0.03**		1.13**	
G x S	11.08 n.s.		0.08 n.s.		3.2 n.s.	

-S, no sulphur applied; +S, sulphur applied; *, **significant at 5% and 1% levels; n.s., no significant difference at $P = 0.05$.

had the lowest SPAD value for leaf greenness while 'Paris Silverskin' had the highest. However, when 5.8 kg S ha^{-1} was supplied, 'White Lisbon' had the highest SPAD

value for leaf greenness while 'Fragrant' had the lowest. Between the two *Allium* spp., *A. fistulosum* had lower average leaf greenness either with or without S application than *A. cepa*. The average SPAD value for leaf greenness of *A. fistulosum* cultivars and the hybrid 'Guardsman' increased by more than 100% when 5.8 kg S ha^{-1} was applied compared with a 65% increase for the *A. cepa* cultivars. There was no significant ($P>0.05$) interaction effect of genotype and S nutrition on leaf greenness.

Leaf production and growth varied between the different genotypes (Table II). Leaf growth increased upon fertilization with S by 19.3, 13.6 and 15.0% for *A. cepa* genotypes, 'Guardsman' (hybrid) and *A. fistulosum* genotypes, respectively. Corresponding increases in green leaf numbers at harvest were 25.8, 17.6 and 11.5%, respectively.

Despite the 100% increase in leaf greenness for *A. fistulosum* genotypes, increases in leaf growth and leaf number show that *A. cepa* cultivars were more responsive to S nutrition and, therefore, more S utilisation efficient. The *A. fistulosum* cultivars Sydney Bunching and Fragrant had the most green leaves at harvest, but were not the tallest plants. The tallest plants were 'White Lisbon' and 'Egyptian Bunching'. The linear association between leaf growth and green leaf number was low ($r = 0.46$; $P = 0.07$; $n = 4$).

Brewster (1990) reported that nutritional disorders, including S deficiency, in bulb onions accelerated maturation and, thereby, terminated leaf production and hastened senescence. Similarly, these effects translated into reductions in leaf production and leaf elongation in spring onion plants that were not supplied with S (Table II). There was no significant ($P>0.05$) interaction of genotype and S nutrition for either leaf production or growth.

Both significant ($P<0.05$) genotypic and interaction of genotype and S nutrition effects were recorded for bulb diameter (Table III). Bulb diameters of 'Paris Silverskin' and 'Guardsman' were not significantly ($P>0.05$) affected by S nutrition. However, S application reduced bulb diameters of the *A. fistulosom* cultivars. Conversely, bulb diameters of *A. cepa* cultivars increased in response to S fertilization.

TABLE III

Bulb diameter, percentage dry-matter content of edible portion and total soluble solids content at harvest after growing for ten weeks of eight spring onion cultivars as affected by genotype and sulphur nutrition

	Bulb diameter (mm)		Dry-matter (%)		Total soluble solids (%)	
Cultivar	-S	+S	-S	+S	-S	+S
Winter Over	14.3	19.4	9.0	10.7	8.0	7.4
Paris Silverskin	17.2	17.0	8.4	10.6	7.7	7.8
White Lisbon	15.8	18.2	10.4	9.6	7.0	7.6
Sydney Bunching	10.3	8.7	13.9	12.8	11.6	8.1
Guardsman	13.3	12.9	10.4	10.6	7.9	6.9
Fragrant	11.6	8.4	12.8	11.2	8.7	7.7
Winter White	13.8	15.4	9.1	10.3	8.2	7.2
Egyptian Bunching	11.4	12.6	12.1	12.3	9.5	8.2
Mean	13.5	14.1	10.8	11.0	8.6	7.6
$LSD_{0.05}$						
Genotype (G)	1.40**		0.12**		0.48**	
Sulphur (S)	0.7 n.s.		0.52 n.s.		0.24**	
G x S	1.94**		1.49**		0.67**	

-S, no sulphur applied; +S, sulphur applied; **significant at 1% level; n.s., no significant difference at $P = 0.05$.

Cultivars Sydney Bunching, Egyptian Bunching and Fragrant had the highest percentage dry-matter (%DM) either with or without S fertilization. With S fertilization, average %DM for the *A. cepa* cultivars increased by 9.2%, but average %DM decreased for the *A. fistulosum* cultivars by 10.4%. %DM content for 'Guardsman' was not affected by S fertilization.

Application of 5.8 kg S ha^{-1} variously increased or decreased TSS levels in the different spring onion genotypes (Table III). A variable effect of S on spring onion TSS agrees with work on bulb onion genotypes (Randle 1992a; 1992b; Randle *et al.*, 1995; Hamilton *et al.*, 1997). For instance, Randle *et al.* (1995) reported a significant ($P<0.05$) interaction between bulb onion genotype and S nutrition for TSS, as reported for spring onions in the present work. High TSS was found in spring onion 'Sydney Bunching' either with or without S application. Nonetheless, application of

S reduced TSS in 'Sydney Bunching' by 30% compared to no reduction in 'Paris Silverskin' and 9% increase in 'White Lisbon'.

The relative increases in leaf greenness for the *A. fistulosum* cultivars following S application did not correspond to changes in leaf production and growth, bulb diameter, %DM and TSS levels. These findings demonstrate that *A. fistulosum* cultivars were relatively inefficient in utilising available soil S compared with *A. cepa* cultivars, as suggested above. A positive linear correlation ($r = 0.64$; $P = 0.01$; $n = 16$) was found between %DM and TSS. However, the strength of this association was cultivar specific. In bulb onions, this association is dependent on non-structural water-soluble solids concentrations in different cultivars (Randle, 1992b). Cultivar-dependent responses to applied S both between and within two spring onion species with respect to leaf greenness (chlorophyll development), green leaf number, leaf length, bulb diameter, DM production and TSS were highly evident in the present work. Similar observations were made by Randle (1992b), who studied 54 bulb onion genotypes.

S nutrition and soil type

Spring onion cultivar 'White Lisbon' grown without addition of S fertilizer showed deficiency symptoms of necrosis, chlorosis (especially of the youngest leaf) and stunted growth. There was a delay in expression of S deficiency symptoms on plants grown without S addition on the clay compared with the sandy loam. This delay may have been due to a relatively high inherent water-soluble sulphate content of the clay compared with the sandy loam (Table I).

On clay and sandy loam soils, leaf greenness and plant height of S deficient (0.0 kg S ha^{-1}) plants were reduced by 64% and 31%, respectively, compared with plants supplied with 2.9 kg S ha^{-1} (Figure 1). Neither leaf greenness nor plant height of 'White Lisbon' were differentially affected by clay or sandy loam soils. Perhaps due to better aeration, fresh weight and dry-matter production were slightly but not significantly ($P>0.05$) higher in plants grown on the sandy loam (Figure 2).

S deficiency reduced fresh weight and DM production on both soil types (Figure 2). Differences in total plant fresh weight and dry-matter production between S deficient and S fertilized plants became evident seven and ten weeks after transplanting into the sandy loam and the clay soils, respectively. At harvest, average fresh weight and dry-matter production were 67% and 75% lower, respectively, in S deficient (0.0 kg S

ha^{-1}) plants grown on both soils compared to those that received either 2.9 or 5.8 kg S ha^{-1}. The lack of gain in fresh weight and DM production when S was increased from 2.9 to 5.8 kg ha^{-1} may represent poor S recovery efficiency (Hawkesford, 2000) within this S fertilizer range.

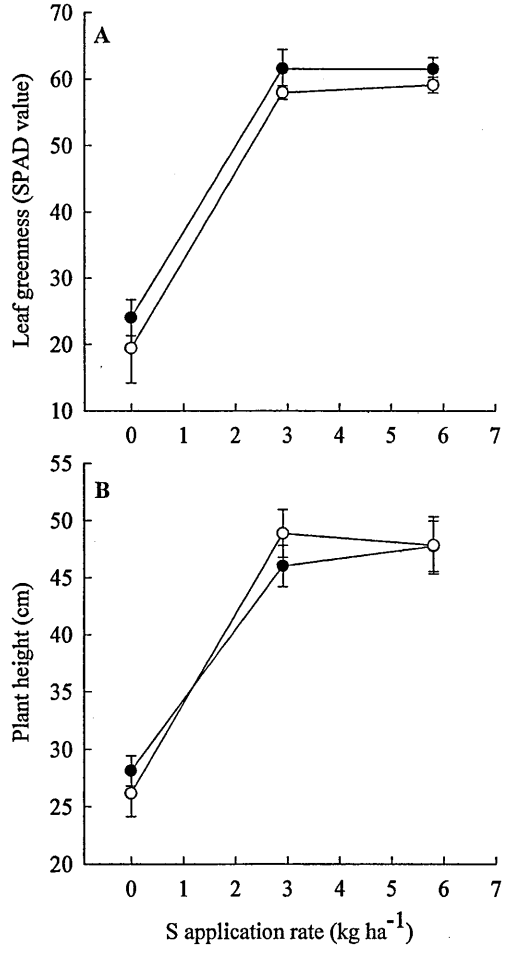

FIG. 1

Leaf greenness (A) and plant height (B) of spring onion cultivar White Lisbon grown on clay (filled circles) and sandy loam (open circles) and supplied with different amounts of sulphur (S). The vertical bars represent standard errors of the

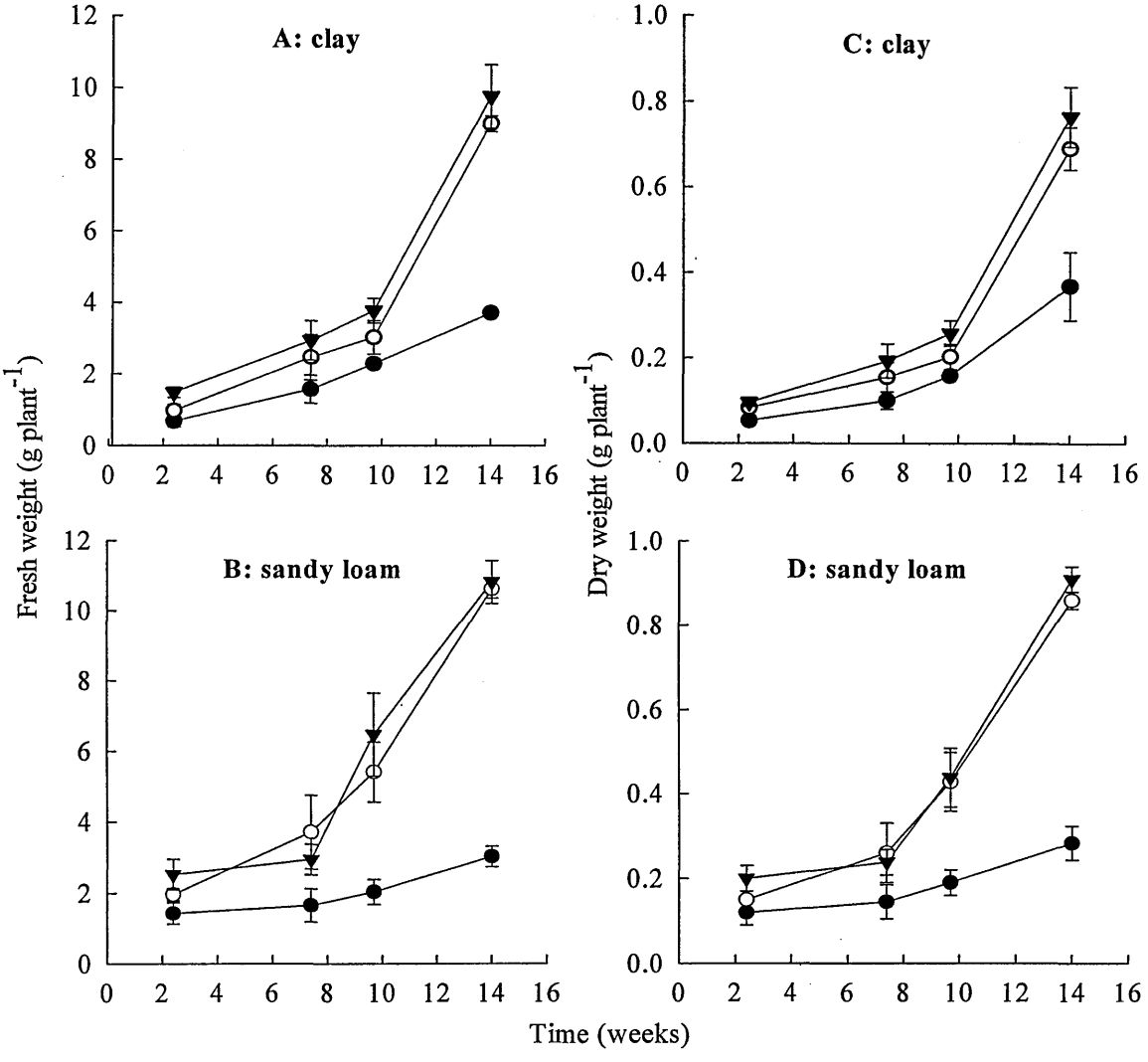

FIG. 2

Fresh weight and dry-matter production of spring onion cultivars 'White Lisbon' grown on clay (A, C) and sandy loam (B, D) at applied sulphur levels of 0.0 (filled circles), 2.9 (open circle) and 5.8 (filled triangle) kg ha^{-1}. The vertical bars represent standard errors of the means (n = 4)

Reductions in root growth due to S stress and the effects of soil type were reflected in reduced shoot growth. Thus, proportionally, shoot and root mass remained in balance (Table IV), as suggested by Brouwer (1962). Accordingly, neither soil type nor S nutrition significantly (P>0.05) influenced this balance.

TSS of 'White Lisbon' decreased with an increase in S fertilizer rate from 0.0 to 2.9 kg ha^{-1}. TSS increased again, but only relatively slightly, when S level was further increased to 5.8 kg ha^{-1} (Table IV). Thus, TSS at 5.8 kg S ha^{-1} was still less than that at the 0.0 kg S ha^{-1} rate. Overall, TSS of plants grown on the sandy loam was higher than on the clay soil, perhaps due to the higher S content of the clay (Table I). Although in contrast to the first experiment, reductions in TSS of 'White Lisbon' with S application agree with a previous report on the same cultivar by Freeman and Mossadeghi (1970). As found in the first experiment, %DM was linearly correlated with TSS content (r = 0.82; P = 0.05; n = 4).

TABLE IV

Shoot/root fresh weight ratio and total soluble solids content at harvest after growing for 16 weeks of spring onion cultivar 'White Lisbon' as affected by soil type and sulphur nutrition

	Shoot:root fresh weight ratio (g g^{-1})		Total soluble solids (%)	
S rate (kg ha^{-1})	Clay	Sand	Clay	Sand
0.0	3.1	2.9	7.6	8.3
2.9	3.3	3.1	6.1	7.1
5.8	3.3	3.3	6.4	7.4
Mean	3.2	3.1	6.7	7.6
$LSD_{0.05}$				
Sulphur (S)	0.56 n.s.		0.29**	
Soil type (soil)	0.46 n.s.		0.24**	
S x soil type	0.79 n.s.		0.41 n.s.	

**significant at 1% level; n.s., no significant difference at P = 0.05.

Conclusion

Variations in responsiveness to S nutrition among different spring onion genotypes were recorded. Spring onion cultivars that achieve optimum growth and yield under low S rates can perhaps be selected in breeding programmes and variety trials for commercial cultivation on inherently low S soils. This proposition is especially

relevant to *A. cepa* cultivars, which increased yield in response to applied S compared with *A. fistulosum* cultivars. Additional S application of 5.8 over 2.9 kg S ha^{-1} was not beneficial, since spring onion 'White Lisbon' growth and dry-matter production were not further enhanced. Fresh weight and dry-matter production were slightly greater on sandy loam compared with clay, possibly due to better aeration. Percentage dry-matter and total soluble solids contents were positively and linearly correlated in spring onions, as found in bulb onions by Darbyshire and Henry (1979).

The authors thank Mr Allen Hilton for his technical advice and assistance.

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4.2 Evaluation of eight spring onion genotypes and sulphur nutrition and soil type effects with an electronic nose

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(Submitted *to Journal of Horticultural Science and Biotechnology*, January 2003)

SUMMARY

Genotype and sulphur (S) nutrition and soil type effects on spring onion quality were assessed using a 32-conducting polymer sensor E-nose. Relative changes in sensor resistance ratio (%dR/R) varied among eight spring onion genotypes. %dR/R was reduced by S application in four of the eight genotypes. For the other four genotypes, S application gave no change in %dR/R in three and increased %dR/R in the other. E-nose classification of headspace volatiles by a two-dimensional principal component analysis (PCA) plot for spring onion genotypes differed for S fertilisation versus no S fertilisation. Headspace volatiles data set clusters for cultivar White Lisbon grown on clay or on sandy loam overlapped when 2.9 (Mahalanobis distance value [D^2] = 1.6) or 5.8 (D^2 = 0.3) kg S ha^{-1} was added. In contrast, clear separation (D^2 = 7.5) was recorded for headspace volatile clusters for 0.0 kg S ha^{-1} on clay versus sandy loam. Addition of 5.8 kg S ha^{-1} increased pyruvic acid content by a factor of 1.72 on average across the eight genotypes. However, increased S from 2.9 to 5.8 kg ha^{-1} did not significantly (P>0.05) influence %dR/R, %DM and TSS contents, but significantly (P<0.05) increased pyruvic acid content. TSS was significantly (P<0.05) reduced by S addition while %DM was unaffected. In conclusion, the 32-conducting polymer E-nose discerned differences in spring onion quality that was attributable to genotype and to variations in growing conditions as shown by the significant (P<0.05)

interaction effects for %dR/R.

Spring onion (*Allium cepa* L.) is valued for distinctive flavours released during chewing (Block, 1992). Flavour in *Allium* spp. is largely comprised of non-protein sulphur (S) amino acid secondary metabolites, which form 1 to 5% of the total tissue dry weight (Freeman and Whenham, 1975; Block, 1992).

Root-absorbed sulphate is reduced to sulphite and assimilated into cysteine, which is used for the syntheses of onion flavour precursors. These precursors are S-methyl cysteine, S-1-propenyl cysteine and S-propyl cysteine sulphoxides (Lancaster and Kelly, 1983; Block, 1992). The flavour precursors are hydrolysed by the enzyme alliinase during tissue disruption to produce both flavour and non-flavour compounds (Block, 1992; Randle *et al.*, 1994; Lancaster *et al.*, 1998; Bacon *et al.*, 1999). Differences in flavour intensity among bulb onion genotypes are associated with variations in the concentration and composition of flavour precursors in the intact tissue (Thomas and Parkin, 1994; Lancaster *et al.*, 1998; Bacon *et al.*, 1999).

Interactions of genotype with growing environment affect onion bulb flavour intensity (Randle *et al.*, 1995; Hamilton *et al.*, 1998; Lancaster *et al.*, 1998; Bacon *et al.*, 1999; Debaene, *et al.*, 1999). Climate and edaphic factors, such as ambient temperature (Abdalla, 1967), soil water content (Gamiely *et al.*, 1991) and soil nutrient status (Freeman and Mossadeghi, 1970; Randle *et al.*, 1995; Hamilton *et al.*, 1998; Lancaster *et al.*, 1998; Bacon *et al.*, 1999), affect growth and quality of bulb and spring onions. The response of bulb and spring onions to S concentration in the growing medium is well recognised. Conventional determinants of bulb onion and spring onion flavour, including lachrymatory factor and thiosulphinate and/or pyruvic acid contents, typically increase with increasing rate of S application (Freeman and Mossadeghi, 1970; Randle *et al.*, 1994; 1995; Hamilton *et al.*, 1997; 1998). Application of 3 meq S l^{-1} (96 mg S l^{-1}) reduced the refractive index of spring onion cultivar White Lisbon juice by 0.4% as compared with no S application (Freeman and Mossadeghi, 1970). Soluble sugar contents in 34 of 69 onion bulb cultivars were also reduced by 12% with the application of 4 meq S l^{-1} (128 mg S l^{-1}) as compared with 0.1 meq S l^{-1} (3.2 mg S l^{-1}; Randle, 1992a). Soluble sugar content of bulb onion genotypes with inherently low bulb dry-matter content is, however, enhanced by increasing S nutrition. Conversely, soluble sugar content is reduced in high dry-matter genotypes (Randle *et al.*, 1995).

Flavour is comprised of specific combinations of complex mixtures of various molecules (Bartlett *et al.*, 1997). Organoleptic tests for flavour determine mutual interactions among various taste and odour compounds, while analytic tests usually assess individual chemicals (Bartlett *et al.*, 1997). Conventional organoleptic and analytic methods for flavour determination are tedious and costly in terms of material inputs and time, especially when evaluating large numbers of samples. Recently, electronic nose (E-nose) sensor technology has been used to discriminate among odours. This method has been reported to be fast, cheap and reproducible (Bartlett *et al.*, 1997). The E-nose transforms complex chemical signatures of headspace volatiles into electrical responses that fingerprint the headspace (Sinesio *et al.*, 2000).

E-nose sensors are classified according to the construction material used. Sensors are usually inorganic crystalline or polycrystalline, organic or polymers, or biological (Gardner and Bartlett, 1999). Polymer sensors derived from pyrrole, aniline and thiophene monomers are normally comprised of between 8 to 48 different sensor sub-unit polymers. The resistance of polymer sensors to the flow of electric current changes reversibly in response to adsorption and desorption kinetics of aroma molecules (Gopel *et al.*, 1998; Gardner and Bartlett, 1999). Sensor response is recorded as relative change in resistance with respect to the original resistance (%dR/R; Benady *et al.*, 1995). Thus, E-noses discriminate the mixture of headspace volatiles in samples presented, but not the individual components (Madsen and Grypa, 2000). Headspace volatiles from agricultural, pharmaceutical and industrial products and medical diagnostic test samples have been successfully discriminated using E-noses with different sensor elements (Bartlett *et al.*, 1997; Di Natale *et al.*, 1997; Madsen and Grypa, 2000; Sinesio *et al.*, 2000). Irreversible binding of S compounds such as those of *Allium* flavours to reactive sites of E-nose sensor elements can have poisoning or inhibitory effects on sensors during volatiles sensing (Payne, 1998; Gardner and Bartlett, 1999). For instance, initial work demonstrated that E-nose sensors could be overwhelmed by onion volatiles, especially S-containing flavour compounds and ammonia immediately on cutting (B. Smith, pers. comm.). This constraint contributes to lack of repeatability of E-nose sensor response data for replicates of the same sample, thus making results unreliable. This phenomenon is termed sensor drift (Payne, 1998).

This study investigates the use of a 32-conducting polymer sensor-based E-nose to qualitatively discriminate among eight spring onion genotypes grown under varied

rates of S nutrition. Also, the effects of S nutrition by soil type on headspace volatiles are reported for one spring onion cultivar.

MATERIALS AND METHODS

Two separate glasshouse experiments were carried out on pot-grown spring onions in the UK between April and December.

Plant material

Seven spring onion genotypes were obtained from the Genetic Resources Unit of Horticultural Research International (HRI), Wellesbourne, UK. These genotypes included four *A. cepa* cultivars (cultivars Winter Over, Paris Silverskin, Winter White Bunching and Egyptian Bunching), two *A. fistulosum* cultivars (cultivars Sydney Bunching and Fragrant) and one *A. cepa* x *A. fistulosum* cross (cultivar Guardsman). Additionally, *A. cepa* cultivar White Lisbon was obtained from a local seed supplier (E. W. King and Co. Ltd., Monks Farm, Colchester, UK) and included in the trial.

Pot filling, planting and fertiliser supply

Clay (Alluvial Gley soil; Thames series, T_S) and sandy loam (Brown Earth; Wick series, WQ_2) soils were dug from research fields at HRI. Plastic pots of 12-cm diameter were bottom-lined with 100 g stone grit and then filled up with 500 g clay (9.9% moisture content on an oven-dry weight basis) or 700 g sandy loam (2.6% moisture content). Spring onion seeds were pre-germinated at 25°C for 5 days in 8.5-cm diameter Petri dishes lined with moistened filter paper before transplanting. The spring onion plants were irrigated with distilled water during their growth. Fertiliser as nutrient solution was applied in split applications 2 and 5 weeks after transplanting, each at half the total recommended rate. Total nutrients applied per pot were 120 mg N (urea, $CO(NH_2)_2$; 106 kg N ha^{-1}), 24 mg P (metaphosphoric acid, H_3PO_4; 33 kg P ha^{-1}), 72 mg K (muriate of potash, KCl; 64 kg K ha^{-1}) and 24 mg Mg (magnesia, MgO; 21 kg ha^{-1}; MAFF, 1994). Application of S in the form of epsom salt (magnesium sulphate, $MgSO_4$) varied from 0 to 6.6 mg per pot (0 to 5.8 kg ha^{-1}).

Genotype and S nutrition experiment

The eight spring onion genotypes were grown on the sandy loam. Plants were supplied with or without epsom salt at the rate of 5.8 kg S ha^{-1}. A randomised

complete block (eight genotypes by ±S nutrition) factorial design with five replications each comprised of two pots per treatment was used. The total number of plants for each treatment was 50 (five plants per pot by two pots per replication by five replicates). The final harvest was 10 weeks after transplanting.

S nutrition and soil type experiment

Spring onion cultivar White Lisbon seedlings were grown in either 500 g clay or 700 g sandy loam. Pots received 0.0, 2.9 or 5.8 kg S ha^{-1}. The experiment design was a randomised complete block factorial (S nutrition by soil type) with three replications. Each replication consisted of 16 pots per treatment. The total plant number for each treatment was 80 (five plants per pot by 16 pots per treatment by three replicates). Final harvest was 15 weeks after transplanting.

E-nose evaluation

Juice from edible portions (i.e. pseudostem plus an equal length of green leaf bases) of harvested spring onion plants were extracted using a Moulinex (Model Tipo 753; Patendo, Spain) juice extractor at room temperature (*ca.* 20°C). After 20 min, 20 ml of 5% trichloroacetic acid (TCA) was added to a 20 ml portion of the homogenate in a 250 ml conical flask and vortexed for *ca.* 15 s. Hydrolysis of flavour precursors by the native alliinase enzyme occurred during the initial 20 min period. To eliminate changes in headspace volatiles composition manifest as sample drift (K. C. Persaud, pers. comm.) during sample preparation, equilibration and headspace gas sensing, the homogenate/TCA mixture was allowed to stand for 1 hr after TCA addition to terminate alliinase activity (Schwimmer and Weston, 1961). Deionised water (10 ml) was added to the homogenate/TCA mixture and vortexed for *ca.* 15 s. A 1 ml aliquot of the diluted solution was put into a 100 ml Schott bottle and capped. The Schott bottle was placed in the sample station of an AromaScan LabStation System (Model A32/8S; Osmetech, UK) to equilibrate at 25°C and 30% RH for 10 min prior to evaluation. Headspace volatiles were sampled for a period of 70 s at a gas flow rate of 50 ml min^{-1}. Percentage change in sensor resistance (Ro - Rs) with reference to a base resistance (Ro) was recorded. That is, %dR/R = (Ro - Rs) / Ro x 100; where Rs is the sensor resistance in the presence of the sample. A completely randomised design with five replications was used. Each of the replicates was comprised of 20 spring onion plants.

Pyruvic acid determination

Total pyruvic acid content was determined on juice extracted from edible spring onion portions. A mixture was made of 1 ml each of 0.0125% (w/v) 2,4-dinitrophenylhydrazine, deionised water and an aliquot of the homogenate/TCA mixture prepared as above. The solution mixture was warmed in a water bath at 37°C for 10 min, after which time 5 ml of 0.6 N NaOH was added and the mix vortexed for *ca.* 15 s. A UV/VIS spectrophotometer (Model PU8730; Unicam, UK) was used to determine absorbance at 420 nm (Schwimmer and Weston, 1961; Randle and Bussard, 1993). A completely randomised design with four replications was adopted with each replicate made up of 20 spring onion plants.

Sensory appraisal of lachrymatory potency

For a check on lachrymatory potency for the Genotype x S nutrition experiment, edible portions of spring onion samples were placed on glass plates and labelled with three digit numbers. The samples were randomly picked and the time to maximum (time-intensity) hotness felt by the tongue during chewing of the spring onions was recorded with a stopwatch (Larmond, 1982). The single appraiser's mouth was rinsed thoroughly with boiled tap water at room temperature between mastication of each sample. Each set of treatment (n = 16) assessments was followed by 20-min break before the next replicate test. A completely randomised design with four replications was adopted for this experiment. Each of the four replicate (r = 4) was comprised of five sample (n = 5) spring onion plants.

Percentage dry-matter and total soluble solids contents

For dry-matter content, 100 g fresh weight of the edible portion was dried in an oven at 60°C to constant weight. Dry-matter content (%) was recorded on a fresh weight basis. For total soluble solids (TSS), edible portions of spring onion were homogenised at room temperature. TSS was determined with a digital refractometer (Model PR1; Atago Co. Ltd., Japan). Three replications were used. Each replicate was made up of five plants.

Data analysis

Proportional changes in resistance ratio (%dR/R) were processed using A32S Microsoft Windows Version 3.24B software (AromaScan Plc., UK). %dR/R data were transformed using the method of square root transformation (Gomez and Gomez, 1984) before ANOVA using Minitab for Windows Version 12.13 (Minitab Inc., USA). Two-dimensional (2D) principal component analysis (PCA) plots were generated for the E-nose sensor response data sets using the AromaScan software. Separations on the PCA plot between data set cluster centres for headspace volatiles for the various treatments were quantified with the A32S software using the minimum Mahalanobis D^2 classification rule. This rule states that an observation 'x' is assigned to a population 'i' if $Di^2<Dj^2$; 'i'≠'j'. $D^2>3.0$ was considered a significant separation between data set clusters (Morrison, 1976; Gnanadesikan, 1977; Mark and Tunnell, 1985). Treatment means for pyruvic acid content, time-intensity of lachrymatory potency, total soluble solids and percentage dry-matter content were separated following ANOVA by the least significant difference (LSD) test at the 5% level.

RESULTS

Genotype and S nutrition

In terms of main factor effects, relative changes in E-nose sensor resistance ratio (%dR/R) of the 32-conducting polymer sensor differed significantly ($P<0.01$) for headspace volatiles among the eight spring onion genotypes (Table 1). In contrast, S fertilisation, as a main factor, did not significantly ($P>0.05$) affect %dR/R. The genotype by S nutrition interaction was therefore significant ($P<0.01$). Individual means showed a significant ($P<0.01$) reduction in %dR/R for three (cultivars Paris Silverskin, Winter White and Egyptian Bunching) of the eight spring onion genotypes upon S addition. In terms of %dR/R, the most responsive cultivar to S addition was Guardsman. Addition of 5.8 kg S ha^{-1} to cultivar Guardsman increased %dR/R from 1.31 at 0 kg S ha^{-1} to 1.69. Addition of S generally tended to reduce %dR/R for both *A. cepa* cultivars (ie. 'Winter Over', 'Paris Silverskin', 'Winter White Bunching' and 'Egyptian Bunching', but not 'White Lisbon') and *A. fistulosum* cultivars (ie. 'Sydney Bunching' and 'Fragrant'). The overall species-based means for %dR/R were greater for *A. fistulosum* cultivars (1.70; $n = 10$) as compared with *A. cepa* cultivars (1.48; $n = 25$) and *A. cepa* x *A. fistulosum* cultivar Guardsman (1.50; $n = 5$).

Table I

Relative change in E-nose sensor resistance (%dR/R), pyruvic acid content, percentage dry-matter and total soluble solids content (TSS) of eight spring onion genotypes grown on sandy loam at 0.0 and 5.8 kg S ha^{-1}

Genotype (G)	%dR/R			Pyruvic acid content (µmole g^{-1} FW)			Dry-matter (%)			TSS content (%)		
	-S	+S	Genotype mean	-S	+S	Genotype mean	-S	+S	Genotype mean	-S	+S	Genotype mean
Winter Over	1.38	1.35	1.37	3.39	6.18	4.79	9.0	10.7	9.9	8.0	7.4	7.7
Paris Silverskin	1.31	1.23	1.27	2.86	6.25	4.56	8.4	10.6	9.5	7.7	7.8	7.8
White Lisbon	1.65	1.68	1.67	5.85	6.65	6.25	10.4	9.6	10.0	7.0	7.6	7.3
Sydney Bunching	1.76	1.74	1.75	4.63	7.60	6.12	13.9	12.8	13.4	11.6	8.1	9.9
Guardsman	1.31	1.69	1.50	3.72	6.20	4.96	10.4	10.6	10.5	7.9	6.9	7.4
Fragrant	1.66	1.63	1.65	3.35	7.47	5.41	12.8	11.2	12.0	8.7	7.7	8.2
Winter White	1.77	1.48	1.63	3.63	6.95	5.29	9.1	10.3	9.7	8.2	7.2	7.7
Egyptian Bunching	1.48	1.43	1.46	4.03	6.73	5.38	12.1	12.3	12.2	9.5	8.2	8.9
Sulphur mean	1.54	1.52		3.93	6.75		10.8	11.0		8.6	7.6	
$LSD_{0.05}$												
Genotype mean (G)	0.026** ($n = 10$)			0.37** ($n = 8$)			0.12** ($n = 8$)			0.48** ($n = 8$)		
Sulphur mean (S)	0.015 n.s. ($n = 40$)			0.18** ($n = 32$)			0.08 n.s. ($n = 32$)			0.24** ($n = 32$)		
G x S	0.037** ($n = 5$)			0.52** ($n = 4$)			0.24** ($n = 4$)			0.67** ($n = 4$)		

-S, no sulphur fertilisation; +S, sulphur fertilisation; **Significant at 1% level; n.s., no significant difference at $P = 0.05$; n, number of observations used for $LSD_{0.05}$ calculation.

Two-dimensional PCA plots showed differences among headspace volatile clusters of the eight spring onion genotypes with (Figure 1A) or without (Figure 1B) S fertiliser application. The spatial distribution pattern of data sets of headspace volatiles for spring onions grown without S addition was different from those that received S fertiliser. Similarities in headspace volatiles were shown by overlaps of data set clusters in the PCA map. These similarities were confirmed by Mahalanobis distance (D^2) values (Table 2). For instance, with no S fertilisation, E-nose data set clusters for cultivars Winter Over versus Egyptian Bunching, Winter Over versus Guardsman or Guardsman versus Egyptian Bunching had $D^2<3.0$. However, significant ($D^2>3.0$) separation among these sets of data clusters of headspace volatiles became evident upon S fertilisation. The location of the headspace volatile cluster for cultivar Winter Over in the PCA map did not change ($D^2 = 0.9$) despite S fertilisation as compared with the other seven cultivars.

Pyruvic acid contents of the eight spring onion genotypes varied with or without S fertiliser application (Table 1). Upon S fertilisation, pyruvic acid contents of cultivars Sydney Bunching and Fragrant were the highest and those of cultivars Winter Over and Guardsman were the lowest. Overall, pyruvic acid content increased by a factor of 1.72 due to S fertilisation. Pyruvic acid content after S fertilisation was greater for *A. fistulosum* cultivars (cultivars Sydney Bunching and Fragrant) than *A. cepa* cultivars (cultivars Winter Over, Paris Silverskin, White Lisbon, Winter White Bunching and Egyptian Bunching) or the *A. cepa* x *A. fistulosum* cross (cultivar Guardsman) as compared with no S fertilisation. A similar trend in variability for genotypic response to S fertilisation was evident in sensory appraisal of lachrymatory (tear-inducing) potency. Lachrymatory potency, as determined by time to maximum intensity of hotness (Larmond, 1982), differed among the eight spring onion genotypes. S fertilisation enhanced lachrymatory potency for 'Guardsman', 'Winter White' and 'Egyptian Bunching' from 82 to 31 ($d = 51$) s, 81 to 27 ($d = 54$) s and 76 to 21 ($d = 55$) s response times, respectively ($n = 20$). The response times for 'Paris Silverskin' (70 to 24 s; $d = 46$ s) and 'White Lisbon' (69 to 30 s; $d = 39$ s) upon S addition were intermediate, while those for 'Winter Over' (58 to 28 s; $d = 30$ s), 'Sydney Bunching' (61 to 29 s; $d = 32$ s) and 'Fragrant' (63 to 32 s; $d = 31$ s) were least.

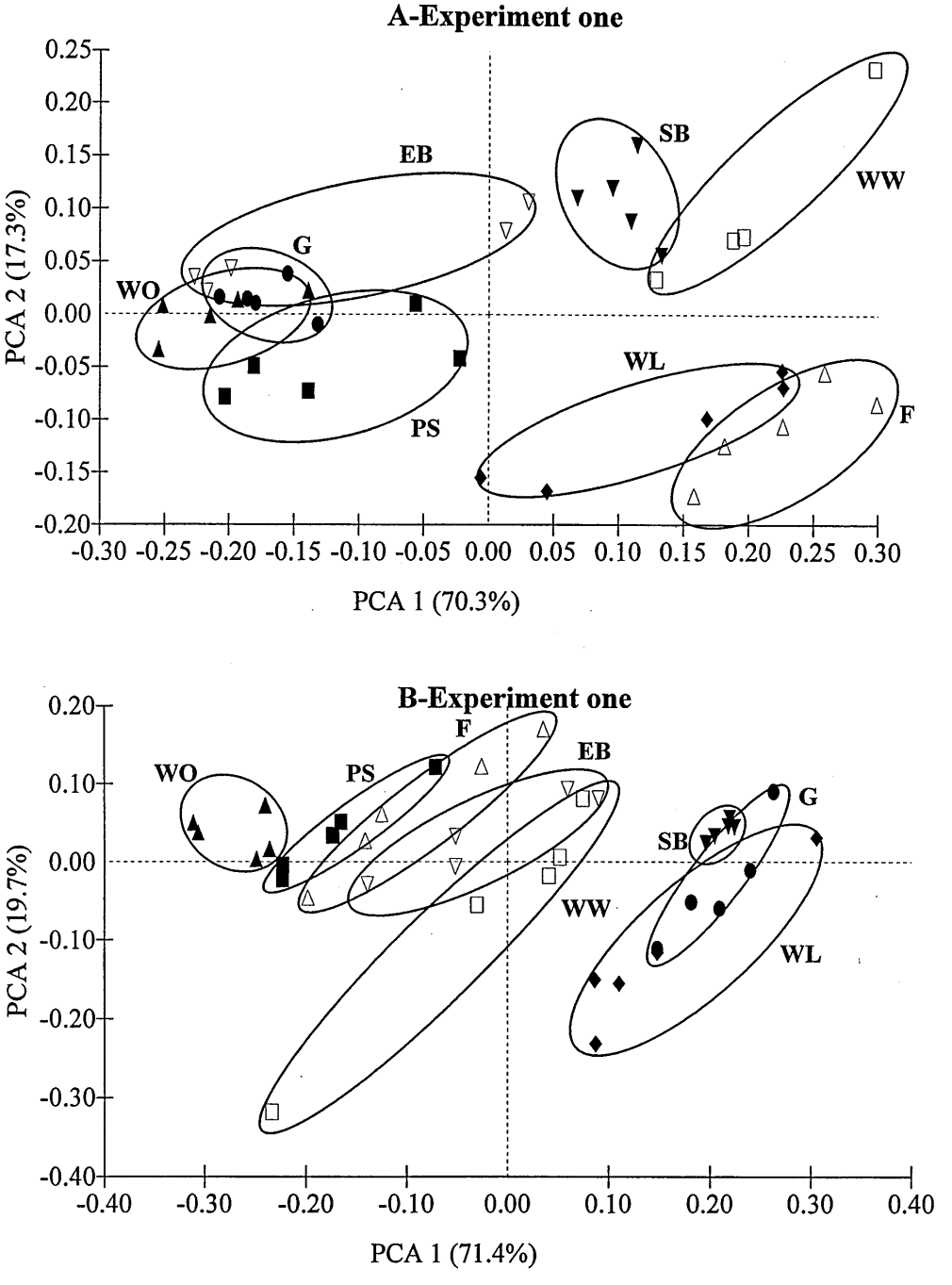

FIG. 1

A 2D PCA plot for E-nose data clusters for headspace volatiles of eight spring onion genotypes grown on sandy loam at S nutrition levels; 0.0 (A) and 5.8 (B) kg S ha^{-1}. Cultivars: WO, Winter Over; PS, Paris Silverskin; WL, White Lisbon; SB, Sydney Bunching; G, 'Guardsman' (hybrid); F, Fragrant; WW, Winter White; EB, Egyptian Bunching ($n = 5$)

Table II

Mahalanobis distance (D^2) values for E-nose data set clusters of headspace volatiles for eight spring onion genotypes grown with or without addition of S fertiliser. Separations between data set clusters are significant at $D^2>3.0$

Genotypes	0.0 kg S ha^{-1}								5.8 kg S ha^{-1}						
	WO	PS	WL	SB	G	F	WW	EB	WO	PS	WL	SB	G	F	WW
0.0 kg S ha^{-1}															
PS	7.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WL	34.5	17.6	-	-	-	-	-	-	-	-	-	-	-	-	-
SB	15.4	7.2	11.3	-	-	-	-	-	-	-	-	-	-	-	-
G	2.4	6.9	26.5	21.1	-	-	-	-	-	-	-	-	-	-	-
F	37.6	20.5	5.2	14.6	31.6	-	-	-	-	-	-	-	-	-	-
WW	24.0	9.8	7.4	6.0	22.5	15.0	-	-	-	-	-	-	-	-	-
EB	2.6	8.0	23.4	5.0	1.1	22.0	8.0	-	-	-	-	-	-	-	-
5.8 kg S ha^{-1}															
WO	0.9	6.2	21.3	24.2	5.4	27.3	20.5	2.4	-	-	-	-	-	-	-
PS	10.2	2.2	31.4	9.6	7.6	30.2	14.5	12.5	5.8	-	-	-	-	-	-
WL	42.2	25.3	11.5	17.2	33.9	5.3	14.1	29.9	27.7	39.1	-	-	-	-	-
SB	51.5	23.7	5.8	17.7	38.6	1.6	26.0	47.4	36.1	71.6	10.0	-	-	-	-
G	43.4	23.4	8.7	17.3	36.7	3.6	21.7	24.3	31.0	34.8	2.4	6.5	-	-	-
F	8.2	3.8	29.1	3.6	6.1	27.4	8.9	4.7	6.3	3.4	36.9	48.2	31.0	-	-
WW	27.4	12.2	6.9	7.5	21.2	13.4	4.0	14.7	17.6	20.6	17.5	18.1	17.7	18.5	-
EB	7.5	6.2	10.4	6.9	15.3	11.9	3.9	22.9	11.4	14.3	18.7	19.7	14.4	12.4	4.5

Cultivars: WO, Winter Over; PS, Paris Silverskin; WL, White Lisbon; SB, Sydney Bunching; G, Guardsman; F, Fragrance; WW, Winter White; EB, Egyptian Bunching.

Percentage dry-matter (%DM) and total soluble solids (TSS) contents were genotypically determined (Table 1). These quality components were also significantly ($P<0.05$) affected by genotype by S fertilisation interaction. Although *A. fistulosum* cultivars Sydney Bunching and Fragrant generally had the highest %DM content, this reduced on average by a factor of 0.10 after S addition in contrast with an increase by a factor of 1.09 for *A. cepa* cultivars Winter Over, Paris Silverskin, White Lisbon, Winter White Bunching and Egyptian Bunching or 1.02 for the *A. cepa* x *A. fistulosum* cross (cultivar Guardsman). Genotypes with high %DM (e.g. cultivars Sydney Bunching, 13.4%; Fragrant, 12.0% and Egyptian Bunching, 12.2%) also had high TSS contents (9.9%, 8.2% and 8.9%, respectively). TSS contents of the spring onions, with the exception of cultivars Paris Silverskin and White Lisbon were significantly ($P<0.01$) reduced by S fertilisation. The proportional reduction in TSS was dependent on genotype in that it varied between 7% ('Winter Over') and 30% ('Sydney Bunching') among the eight spring onion genotypes.

S fertilisation and soil type

In agreement with results for the genotype and S nutrition experiment, %dR/R for spring onion cultivar White Lisbon did not differ significantly ($P>0.05$) for different S fertiliser rates (Table 3). The interaction of S nutrition by soil type for %dR/R was significant ($P<0.05$). Under no S fertilisation, spring onions grown on sandy loam had the greatest ($P<0.05$) %dR/R, while those grown on the clay had the smallest %dR/R. %dR/R significantly ($P<0.05$) reduced from 1.40 to 1.28 following an increase in S rate from 0 to 5.8 kg S ha^{-1} on the sandy loam. For plants grown on the clay, %dR/R significantly ($P<0.05$) increased from 1.22 to 1.35 following application of 2.9 kg S ha^{-1}. There was a significant ($P<0.05$) reduction in %dR/R from 1.35 to 1.23 on the clay when S fertiliser was further increased from 2.9 to 5.8 kg ha^{-1}. Overall, %dR/R was significantly ($P<0.05$) greater for headspace volatiles of spring onions grown on the sandy loam compared with those for the clay.

Under limited S (0.0 kg ha^{-1}) nutrition, headspace volatiles for spring onion cv. White Lisbon grown on the clay were widely separated from those for the sandy loam in 2D PCA map with a large D^2 value of 7.5 (Figure 2; Table 4). Headspace volatiles of spring onions grown on the clay overlapped with those grown on the sandy loam at each of the S fertiliser rates of 2.9 and 5.8 kg ha^{-1}.

Table III

Relative change in E-nose sensor resistance (%dR/R), pyruvic acid content, percentage dry-matter and total soluble solids content (TSS) of spring onion cultivar White Lisbon grown on clay and sand loam at 0.0, 2.9 and 5.8 kg S ha^{-1}

S rate (kg ha^{-1})	%dR/R			Pyruvic acid (µmole g^{-1} FW)			Dry-matter (%)			TSS (%)		
	Clay	Sand	S mean	Clay	Sand	S mean	Clay	Sand	S mean	Clay	Sand	S mean
0.0	1.22	1.40	1.31	6.33	5.32	5.83	10.2	9.6	9.9	7.6	8.3	8.0
2.9	1.35	1.31	1.33	10.61	12.09	11.35	9.0	8.4	8.7	6.2	7.1	6.7
5.8	1.23	1.28	1.26	13.78	13.32	13.55	8.4	9.0	8.7	6.4	7.4	6.9
Soil mean	1.27	1.33		10.24	10.24		9.0	9.0		6.7	7.6	
$LSD_{0.05}$												
S mean	0.046 n.s. ($n = 10$)			0.312** ($n = 8$)			0.30 n.s. ($n = 8$)			0.29** ($n = 8$)		
Soil mean	0.034* ($n = 15$)			0.255 n.s. ($n = 12$)			0.24 n.s. ($n = 12$)			0.24** ($n = 12$)		
S x soil	0.059* ($n = 5$)			0.442** ($n = 4$)			0.42 n.s. ($n = 4$)			0.41 n.s. ($n = 4$)		

*, **Significant at 5% and 1% levels; n.s., no significant difference at $P = 0.05$; n, number of observations used for $LSD_{0.05}$ calculation.

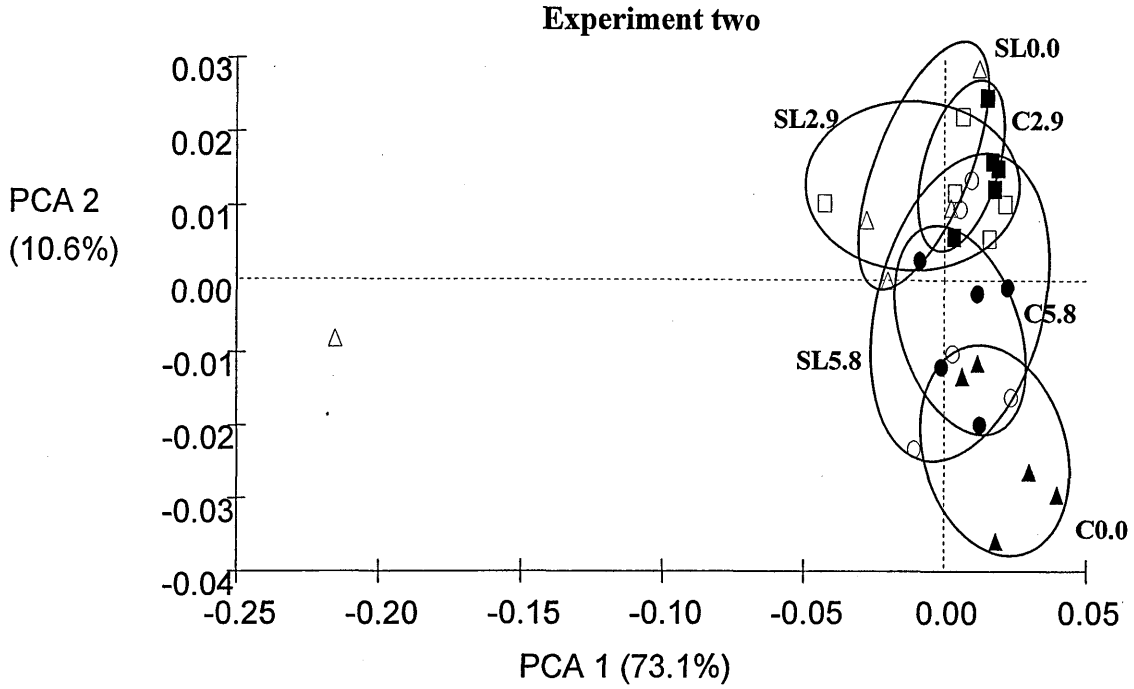

FIG 2

A 2D PCA plot for E-nose data clusters for headspace volatiles of spring onion cultivar White Lisbon grown on clay (C) and sandy loam (SL) at S nutrition levels of 0.0, 2.9 and 5.8 kg ha^{-1} ($n = 4$)

TABLE IV

Mahalanobis distance (D^2) values for E-nose data set clusters of headspace volatiles for spring onion cultivar White Lisbon grown on two different soil types at three different S fertiliser rates. Separations between data set clusters are significant at $D^2>3.0$

S rate (kg ha^{-1})	*Clay soil*			*Sandy loam soil*	
	0.0	2.9	5.8	0.0	2.9
Clay soil					
2.9	8.7	-	-	-	-
5.8	3.5	5.3	-	-	-
Sandy loam soil					
0.0	7.5	1.9	4.6	-	-
2.9	8.2	1.6	4.7	1.5	-
5.8	3.3	3.3	0.3	3.4	2.9

Headspace volatiles of plants grown on sandy loam at 0.0 kg S ha^{-1} were similar to those supplied with 2.9 kg S ha^{-1} on both soil types. Separation between E-nose data

set clusters for spring onions that received 2.9 kg S ha^{-1} versus 5.8 kg S ha^{-1} was significant (D^2>3.0).

Pyruvic acid contents of spring onion cv. White Lisbon grown on clay or on sandy loam were significantly (P<0.01) increased by a factor of 1.95 following application of 2.9 kg S ha^{-1} fertiliser (Table 3). Further addition of S fertiliser from 2.9 to 5.8 kg ha^{-1} increased pyruvic acid content by a much smaller margin of 1.19-fold. For pyruvic acid content, the interaction of S nutrition by soil type was significant (P<0.01). For instance, the greatest increase in pyruvic acid content was recorded for spring onions grown on the sandy loam with S applied at 2.9 kg ha^{-1} compared with the same soil type that was not S fertilised. Differences in soil type did not affect pyruvic acid content of the spring onions.

%DM accumulation in spring onion cv White Lisbon was not significantly (P>0.05) affected by S fertilisation, soil type or S nutrition by soil type interaction (Table 3). TSS of spring onions that received 2.9 and 5.8 kg S ha^{-1} were significantly (P<0.01) reduced from 8.0% (0.0 kg S ha^{-1}) to 6.6 and 6.9%, respectively (Table 3). Thus, application of 5.8 kg S ha^{-1} did not further decrease TSS content beyond that caused by application of 2.9 kg S ha^{-1}.

DISCUSSION

E-nose sensor conductivity and distribution pattern of data sets

The differential response of the E-nose conducting polymer sensor to the spring onion headspace volatiles of the different genotypes was apparently due to variations in composition and concentration of headspace volatile compounds (Randle *et al.*, 1995; Bacon *et al.*, 1999). It may be argued that the time lapse of 1 hr 30 min between homogenisation and E-nose evaluation can allow appreciable loss in onion flavour volatiles, especially lachrymatory factor thiopropanal S-oxide (Block, 1992; Randle, 1997). However, preliminary work (data not presented) showed that when onion bulb samples were presented to E-nose sensor elements immediately after cutting or homogenisation the results were not reproducible. Significantly (P<0.05) large variations within replicates of the same sample were associated with sensor drift. In this circumstance, sensor drift can be attributed to changes in chemical compounds in the homogenate; i.e. sample drift (Block, 1992; Randle *et al.*, 1994; Lancaster *et al.*, 1998; Bacon *et al.*, 1999). Analytical tests for different *Allium* types using HPLC, NMR and GC-MS did not show changes in thiosulphinates when the time span

between homogenisation and extraction was extended to 6 hr (Block, 1992). Thus, variations in headspace volatiles detected by the E-nose can be ascribed to genetic factors and the significant ($P<0.01$) interaction effect of spring onion genotype by S nutrition.

E-nose sensor conductivity following S fertilisation on average was more reduced (large %dR/R value) by headspace volatiles of *A. cepa* x *A. fistulosum* cultivar Guardsman than by those of either *A. cepa* (cultivars Winter Over, Paris Silverskin, White Lisbon, Winter White Bunching and Egyptian Bunching) or *A. fistulosum* (cultivars Sydney Bunching and Fragrant). Differences in edaphic characteristics of clay and sandy loam also influenced spring onion cultivar White Lisbon headspace volatiles and, therefore, E-nose sensor response. The differential responses of the E-nose sensors were clearly represented by data set cluster separations in the 2D PCA plots (Figures 1 and 2). The statistical significances of these separations were confirmed by Mahalanobis distance values of $D^2>3.0$ (Tables 2 and 4). The two PCA scores, PCA 1 (X-coordinate) plus PCA 2 (Y-coordinate) explained over 80% of the total variance for each PCA plot (Figures 1 and 2).

At 0.0 kg S ha^{-1}, relative location of E-nose data set cluster of headspace volatiles for the spring onions was dependent on inherent soil nutrient status since all other stress factors were controlled (Figure 2). Thus, the large D^2 value of 7.5 for the 0.0 kg S ha^{-1} treatment could be attributed to inherent differences in edaphic factors such as water-soluble sulphate content between the clay (63 mg kg^{-1} air dry soil) and the sandy loam (41 mg kg^{-1} air dry soil). Other subtle differences in soil characteristics (Lambers *et al.*, 1998) could also contribute to the large D^2 value. The headspace volatile fingerprints for a specific rate of S fertilisation for the different soil types, overlapped. Thus, headspace volatiles of spring onion cultivar White Lisbon grown on the clay overlapped with those grown on the sandy loam when 2.9 ($D^2 = 1.6$) or 5.8 ($D^2 = 0.3$) kg S ha^{-1} was supplied compared with 0.0 ($D^2 = 7.5$) kg S ha^{-1} treatment.

Pyruvic acid content

The index of flavour, pyruvic acid content of spring onion genotypes increased with application of 5.8 kg S ha^{-1} from 0.0 kg S ha^{-1}. However, relative increase in this flavour index varied among the eight genotypes. Genetic regulation of excessive accumulation of S and poor S-utilisation efficiency by plants (Hawkesford, 2000) could explain the small percentage increase in pyruvic acid content by a factor of 1.19

after increasing S from 2.9 to 5.8 kg ha^{-1}. Differences in soils also contributed to differences in lachrymatory potency, but did not influence pyruvic acid content.

Percentage dry-matter and total soluble solids contents

The existence of a strong positive linear correlation between %DM and TSS for the spring onion genotypes (coefficient of correlation = 0.64; P = 0.01; n = 16) is in agreement with data for onion bulb genotypes (Darbyshire and Henry, 1979). Although there were genotypic differences, the average %DM and TSS for the *A. fistulosum* (cultivars Sydney Bunching and Fragrant) were higher than for *A. cepa* (cultivars Winter Over, Paris Silverskin, White Lisbon, Winter White and Egyptian Bunching). The average DM was 13% for *A. fistulosum* and 10% for *A. cepa,* while TSS values were 9% and 8%, respectively. The significant (P<0.01) reductions in TSS due to S fertilisation can be attributed to reductions in %DM with S fertilisation although not significantly (P>0.05). This association agrees with other studies on spring onion (Freeman and Mossadeghi, 1970) and bulb onion (Randle 1992a; 1992b; Hamilton *et al.*, 1997). A reduction in soluble sugar concentrations of onion bulbs when S nutrition is increased has been associated with lowering of non-structural water-soluble carbohydrate content (Randle, 1992a; 1992b). This effect may explain reductions in TSS in the present study following S fertiliser addition to the growing medium.

Conclusion

The present work shows that spring onion flavour and headspace volatile compounds are largely affected by genotype, S nutrition, and soil type; and by their interaction. Variations in spring onion headspace volatiles due to genotypic and edaphic factors can be assessed by relative changes in E-nose conducting polymer sensor resistance ratios (%dR/R) and principal component analysis (PCA) plots. Responses of spring onion genotypes to S fertiliser supply differed. *A. cepa* x *A. fistulosum* cultivar Guardsman was the most responsive cultivar to S application, with a 1.29-fold increase in %dR/R and a D^2 of 36.7 as compared with *A. cepa* cultivar Winter Over (D^2 = 0.9). Increasing S fertiliser rate reduced the extent of variation in headspace volatile characteristics of spring onion cultivar White Lisbon grown on clay versus sandy loam. That is, it reduced differences evident when S fertiliser was not applied. The E-nose could discriminate, but differences were not associated with

pyruvic acid content; which is a generally accepted method for assessing onion flavour. Further work is required to identify individual or groups of chemical compounds in the spring onion headspace volatiles that interact with the E-nose sensor.

The authors thank Charles Marshall for advice on statistical analysis and Allen Hilton for technical assistance.

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4.3 WATER-DEFICIT STRESS AND SOIL TYPE EFFECTS ON SPRING ONION GROWTH AND HEADSPACE VOLATILES EVALUATED USING AN ELECTRONIC NOSE

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(Submitted to *Journal of Vegetable Crop Production*, July 2003)

ABSTRACT

Effects of water-deficit stress and soil type on growth and flavour intensity of spring onion (Allium cepa L.) cv. White Lisbon and the use of an electronic nose (E-nose) to discriminate between treatments were investigated. Plants were grown in a glasshouse in pots containing clay (Alluvial Gley) or sandy loam (Brown Earth). Irrigation regimes were regular watering to near field capacity (-0.01 MPa) or re-watering to near field capacity when the available soil moisture level was depleted to either ≤50% (-0.80 MPa) or ≤25% (-1.19 MPa). Regular watering significantly ($P<0.05$) increased relative water content and leaf water potential. Periodic water-deficit stress increased leaf number, but reduced leaf length. At -0.01 MPa, plant fresh weight and dry matter content were increased, especially for plants grown on clay, as compared with the -0.80 or -1.19 MPa treatments. Pyruvic acid content for -0.01 MPa on clay was 57% higher than for sandy loam. Lachrymatory potency significantly ($P<0.01$) increased on clay as compared with sandy loam, but was not affected by water-deficit levels. E-nose sensor response (%dR/R) reduced with increasing water-deficit, but was not affected by soil type. Increasing water-deficit reduced separations by three dimensional principal component analysis between headspace volatile data set clusters for plants grown on clay but not on sandy loam. The E-nose had demonstrated potential for discrimination of spring onion quality.

However, further detailed work is required to characterise and optimise interactions of spring onion volatile components with conducting polymer E-nose sensor elements.

Keywords: Allium, clay, flavour, growth, sandy loam, soil water potential

INTRODUCTION

Spring onion (Allium cepa L.) is a tasty salad vegetable that is consumed worldwide due to volatile flavours released during tissue disruption (Block, 1992). Quality in fresh produce is determined by many different preharvest and postharvest factors (Joyce, 1999). Growth, yield and quality of A. cepa are generally dependent on genetic, environment and management factors (Gamiely et al., 1991; Hussaini and Amans, 2000; Shock et al., 2000).

Onion growing seasons are often marked by high evapotranspiration and low rainfall input, making irrigation important. Fluctuations in soil moisture content can change soil structure, which affects soil aeration and soil water and nutrient availability to plants (Rowell, 1996). Consequently, the physiological activity of the root system can be affected (Hsiao, 2000). Changes in growth due to water stress may also influence plant source:sink ratios. Shoot growth is inhibited by water-deficit stress, whereas root growth is either less affected or even stimulated under the same conditions (Brouwer, 1963; Creelman et al., 1990; Hsiao, 2000).

Regular watering increases onion bulb flavour intensity (Gamiely et al., 1991), presumably by enhancing soil nutrient uptake and plant metabolism. Other reports for bulb onion (Kratky et al., 1990) and crop plants in general (Beverly et al., 1993) suggest an increase in assimilate production and total soluble solids content by mild water-deficit stress. Darbyshire and Henry (1979) indicated that fructans might be hydrolysed to fructose to assist in maintenance of tissue water potential when onion bulb absorbs excessive water. However, the increased water uptake may have a dilution effect on composite chemicals, thereby reducing onion flavour as measured by pyruvic acid and soluble sugar concentrations (Hamilton et al., 1998).

Soil physical structure and texture properties influence water and mineral nutrient availability to plant roots (Beverly et al., 1993; Rowell, 1996). For instance, growth and yield of bulb onions on loamy or on clay soils were not affected when they were re-watered to near field capacity following depletion by 20% and 40% of the

available water-holding capacity, respectively (Talha et al., 1978). However, growth and yield of bulb onions on loose sand were reduced even under regular irrigation as compared to sandy loam or clay. This differential effect may be explained by the poor capillarity, easy leaching of nutrients, large air spaces and poor water retention capacity of loose sand soil (Rowell, 1996).

Biochemical determination of pyruvic acid or thiosulphinate concentration and/or organoleptic appraisal of lachrymatory (tear-inducing) potency (Freeman and Whenham, 1975) are conventionally used to measure onion flavour. However, these methods are difficult, expensive and time-consuming; especially when evaluating large numbers of samples. The electronic nose (E-nose) is a relatively novel device used for qualitative volatiles sensing. This device incorporates inorganic crystalline or polycrystalline, organic or polymer, and biologically derived (Gardner and Bartlett, 1999) sensors that interact with molecules of headspace gas. For multiple unit polymer sensors derived from pyrrole, aniline and thiophene monomers, these interactions cause changes in sensor resistance to electron flow. As a result, an electronic fingerprint of the odour can be produced (Gopel et al., 1998).

Principal component analysis (PCA) maps are commonly used in association with E-nose technology for classification of headspace volatiles from agricultural, health, pharmaceutical and industrial products (Persaud and Talou, 1996; Bartlett et al., 1997; Sinesio et al., 2000). For muskmelon and tomato, respectively, Benady et al. (1995) and Sinesio et al. (2000) were able to interpret PCA maps of headspace volatiles data in terms of flavour. Smaller changes in sensor resistance have been associated with higher flavour intensity of muskmelon (Benady et al., 1995). Abbey et al. (2001) discriminated amongst five Allium types using a 32-conducting polymer sensor E-nose. Variable sensor response to the five different Allium types may be attributed to differences in flavour intensity and, perhaps, other non-flavour related headspace volatiles.

Most studies on soil type and physiological stress effects on growth and quality of Allium spp. have focused on bulb onions. This study investigates the effects of water-deficit stress and soil type on growth and dry-matter production of spring onion cv. White Lisbon. It also investigates the potential application of a conducting polymer sensor E-nose to differentiate among spring onions as affected by water-deficit stress and soil type.

MATERIAL AND METHODS

Plant material - Seeds of spring onion cv. White Lisbon were pre-germinated at 25°C in Petri dishes. Five seedlings were transplanted 5 days after germination into 12-cm diameter plastic pots in late summer.

Soil physical and chemical properties - The soils used were clay (Alluvial Gley; Thames series, T_s) and sandy loam (Brown Earth; Wick series, WQ_2). The clay was dug from a fallowed field and the sandy loam from a continuously cropped field at Horticultural Research International (HRI), Wellesbourne (UK). The soils were air-dried to moisture contents of 9.9% for the clay and 2.6% for the sandy loam on an oven dry weight basis. The air-dried soils were passed through a 2-cm^2 mesh sieve. Sand table and pressure membrane methods (Hess and Lovelace, 1998) were used to determine water release characteristics of the soils. Water-soluble sulphate (1:5 soil/water extract) and nitrogen content using the Kjehdal method were also determined on the air-dry soils (Table 1).

TABLE 1.

Some physical and chemical properties of clay and sandy loam soils used in this study of spring onion growth and quality

Parameter	Soil type	
	Clay	Sandy loam
Particle density (g cm^{-3})	2.1	2.5
Dry bulk density (g cm^{-3})	1.1	1.4
Water-soluble sulfate (mg kg^{-1})	62.6	40.6
N (g kg^{-1})	68.7	31.6
Water content (mL) at soil water potential:		
-0.01 MPa	196.5	104.4
-0.80 MPa	168.5	75.5
-1.19 MPa	154.5	61.1

Pot filling and nutrient supply - Pots contained either 500 g clay or 700 g sandy loam on top of 100 g washed stone grit to improve drainage and aeration. The soils were

compacted to fill approximately 500 cm^3 of the pots and were watered using distilled water to field capacity 48 hr prior to transplanting. Fertiliser in the form of nutrient solution was supplied in two equal splits at 2 and 5 weeks after transplanting. Total fertiliser rates were 120 mg N, 24 mg P, 72 mg K, 24 mg Mg and 96 mg S per pot (MAFF, 1994). Plants were watered with distilled water during growth.

Experiment treatments and design - Irrigation treatments were regular watering to near field capacity (-0.01 MPa soil water potential, SWP) and re-watering to near field capacity when soil moisture content was either ≤50% (-0.80 MPa SWP) or ≤25% (-1.19 MPa SWP) of available water holding capacity. Changes in plant fresh weight were determined every 2 weeks by destructive sampling from extra pots. The total plant fresh weight was then subtracted from the total pot weight to estimate water content of the growing medium on a daily basis. Total amounts of water supplied during the 12 weeks of growth were 11.8, 10.1, and 8.6 L per pot grown on clay and 9.2, 7.6 and 6.5 L per pot grown on sandy loam for -0.01, -0.80 and -1.19 MPa SWP levels, respectively. A randomised complete block factorial design was adopted. There were three replicates of each treatment combination. Each replicate was comprised of 12 pots, with 5 plants per pot. Thus, there were 60 plants for each replicate of each treatment. Final harvest was carried out 12 weeks after transplanting.

Preharvest data

Relative water content, leaf water potential and water-use efficiency - Relative water content (RWC) and leaf water potential (LWP) of the 5th or 6th oldest leaf were determined before and after 24 hr from re-watering to near field capacity at 9 weeks after transplanting. RWC was determined using the method of Slavik (1974) with slight modification. A 2-cm length of an approximately 4-mm diameter tissue was cut from the middle leaf portion. Its fresh weight (FW) was quickly determined. The tissue was cut longitudinally on one side, spread and floated inside down on distilled water. The leaf tissue was weighed hourly until constant weight was attained. Saturated weight (SW) was recorded after blotting dry the leaf surface with Whatman No. A1 filter paper. Dry weight (DW) was recorded after drying to constant weight in

an oven for 24 hrs at 45°C. RWC was calculated using the equation: (FW-DW)/(SW-DW) x 100.

LWP was determined using a pressure chamber (Turner, 1988) at 9 weeks after transplanting. Leaves were wrapped in polythene immediately after excision. The leaf stem-end was inserted into a rubber gland greased with silicone. The pressure chamber unit was sealed and nitrogen pressure was steadily increased at a rate of 0.03 MPa s^{-1}. LWP was recorded from the pressure gauge just as water exuded from the cut surface.

For water-use efficiency (WUE), transpiration was determined from the difference between evapotranspiration (weight loss from the total weight of pots plus plants) and soil evaporation (weight loss from the same quantity of soil in pots without plants): WUE = dry-matter (DM)/transpiration (Ts) (Lambers et al., 1998).

Leaf length, number of green leaves, plant fresh weight and dry-matter content - Leaf length was measured from the tip of the longest leaf down to the point of attachment of the leaf blade to the pseudostem at harvest. The number of green leaves per plant, including newly emerged ≥1-cm long leaves was recorded at harvest. Total plant fresh and dry weights were determined during growth by destructive sampling every 2 weeks after transplanting. Edible portion (pseudostem plus equal length of green leaves), the remaining parts of the foliage and the roots were weighed separately immediately after harvest. After recording the fresh weights, the spring onion tissues were dried in an oven at 60°C to constant weight.

Postharvest data

The edible portions of spring onion plants from all three replicates (12 pots each) were combined and then sub-sampled into either four replicates comprised of 12 plants or, in the case of E-nose evaluation, five replicates of 15 plants. The edible portions were homogenised in a Moulinex (Tipo 753; Patendo, Spain) mixer at room temperature.

E-nose discrimination - For E-nose evaluation, a mixture of homogenate and 5% trichloroacetic acid (TCA) (1:1 v/v) was prepared. TCA was added 20 min after homogenisation to stop the activity of the enzyme alliinase (Schwimmer and Weston,

1961) in order for the headspace flavour volatiles to stabilise. The homogenate/TCA solution was diluted with deionised water (1:1 v/v) and vortexed for 15 s. One mL of the diluted solution was put into a 100 mL Schott bottle. The bottle was placed in the sample station of an AromaScan LabStation System A32/8S (Osmetech, Crewe, UK) to equilibrate for 10 min at 25°C and 30% RH before sampling. Headspace gas was sampled for a period of 70 s at a gas flow rate of 50 mL min^{-1} at 25°C and 30% RH. The response of the 32-conducting polymer sensor to headspace volatiles of the mixture for each treatment sample was analysed and processed using A32S Microsoft Windows Version 3.24B software (AromaScan Plc., UK). A three-dimensional (3D) principal component analysis (PCA) plot was also generated. Eigenvalues (variances) were calculated by multiplying the fraction of explained variance by the number of variables (Wold et al., 1987). Separations between centres of treatment data set clusters on the PCA plot were determined with A32S software by the Mahalanobis distance (D^2). A D^2 value greater than three standard deviations (i.e. $D^2>3.0$) was considered as significant separation between two data set clusters on the 3D PCA plot (Mark and Tunnell, 1985).

Pyruvic acid content, lachrymatory potency and total soluble solids content - To determine pyruvic acid content, 1 mL each of 0.125% 2,4-dinitrophenylhydrazine (2,4-DNPH) and deionised water were added to a 1 mL aliquot of homogenate/TCA solution and vortexed for 15 s. This solution was warmed in a water bath at 37°C for 10 min. Five mL of 0.6 N NaOH was then added and vortexed for 15 s. An UV/VIS spectrophotometer (Model PU8730; Unicam, UK) was used to measure absorbance at 420 nm (Randle and Bussard, 1993).

Edible portions of spring onion samples were placed on glass plates and labelled with three digit numbers. The samples were randomly picked and the time to maximum lachrymatory potency (time-intensity of hotness) felt by the tongue during chewing (Larmond, 1982) of the spring onions was measured with a stopwatch. The appraiser's mouth was rinsed thoroughly with boiled tap water at room temperature between mastication of each sample. Each test was followed by 20 min break before the next.

Total soluble solids (TSS) content of homogenised edible portion was determined with Atago digital refractometer (Model PR1; Atago Co. Ltd., Tokyo, Japan).

Analysis and graphing - The E-nose sensor response (%dR/R) data sets were transformed before ANOVA by the square-root transformation rule (Gomez and Gomez, 1984) using Minitab for Windows Version 12.23 software (Minitab Inc., Pennsylvania, USA). ANOVAs for balanced designs were performed on RWC, LWP, WUE, green leaf number, leaf length, plant fresh weight, dry-matter, pyruvic acid content, T-I and TSS data. Data for RWC and number of green leaves were transformed using arcsine and logarithm functions, respectively, before ANOVA (Gomez and Gomez, 1984). However, for convenience of interpretation, original untransformed data are presented. The least significance difference (LSD) method was used to separate treatment means at P=0.05. SigmaPlot Version 5.0 (SPSS Inc., California, USA) was used to plot graphs and trend lines.

RESULTS AND DISCUSSION

Relative water content, leaf water potential and water-use efficiency - Spring onion leaf tissue RWC measured before re-watering to near field capacity was significantly ($P<0.05$) affected by water-deficit stress, soil type and their interaction. RWC just before irrigation was significantly ($P<0.05$) higher for regular irrigation at -0.01 MPa, especially on the clay soil (Fig. 1A). Severe water deficit at -1.19 MPa significantly ($P<0.05$) reduced RWC, especially for plants grown on the sandy loam. After 24 hr from irrigation, RWC increased in all the plants irrespective of treatment, except for the -0.01 MPa clay soil (Fig. 1B). On average across soil types, increases in RWC at -0.01, -0.80 and -1.19 MPa 24 h after irrigation were 3, 6 and 14%, respectively. Thus, increases were proportionally greater for water-deficit stressed plants.

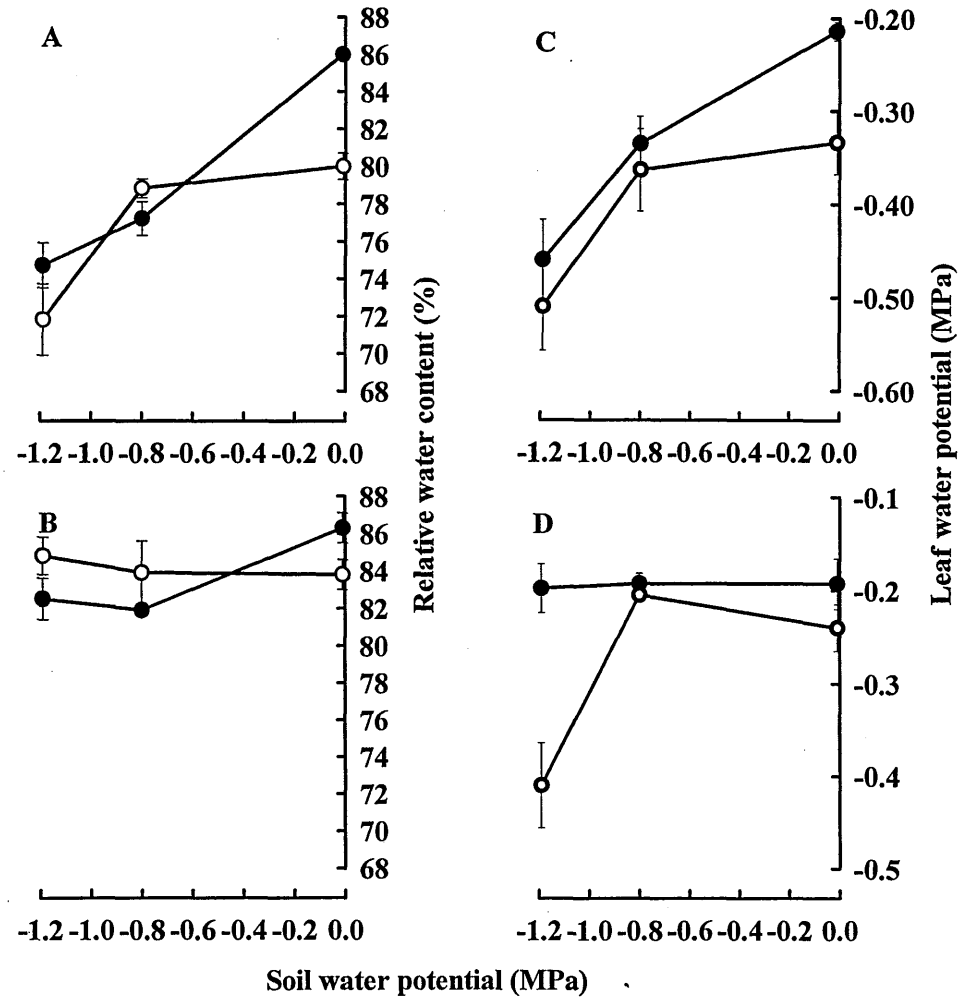

Fig. 1. Relative water content (A, B) and leaf water potential (C, D) of spring onion cv. White Lisbon grown on clay (filled circle) and on sandy loam (open circle) at different soil water potential levels before (A, C) and after (B, D) 24 h of irrigation. The vertical bars represent standard error of the means (n = 16).

LWPs measured prior to irrigation were similar for the clay and sandy loam at -0.80 or -1.19 MPa, but significantly ($P<0.05$) lower on the sandy loam compared with the clay at -0.01 MPa (Fig. 1C). However, 24 h after irrigation, LWP was similar for -0.01 and -0.80 MPa plants on both the clay and sandy loam soils, but higher for -1.19 MPa plants on the clay than on the sand (Fig. 1D). That is, high LWP of ca. -0.20 MPa was recorded for all the plants except the most stressed plants at -1.19 MPa on the sandy loam, for which the LWP was -0.41 MPa 24 h after irrigation. Before re-watering to near field capacity, LWPs of stressed plants ranged between -0.34 and

-0.46 MPa for clay and -0.36 and -0.51 MPa for sandy loam. A decrease in LWP from -0.20 to -0.55 MPa has been reported to reduce relative growth rate of onion leaves (Brewster, 1990).

TABLE 2.

Water-use efficiency (WUE), shoot:root (S:R) fresh weight ratio, percentage change in electronic nose sensor resistance (%dR/R), pyruvic acid concentration, time-intensity of lachrymatory potency (T-I) and total soluble solids content (TSS) of spring onion cv. White Lisbon grown on clay or sandy loam at three water-deficit stress levels

SWP (MPa)	WUE (g DW mL^{-1})	S:R ratio	%dR/R	Pyruvic acid (μmole g^{-1} FW^{-1})	T-I (s)	TSS (%)
Clay						
-0.01	0.7±0.04a	2.7±0.31a	1.60±0.012a	7.77±0.30a	29±3.4a	9.9±0.05a
-0.80	0.6±0.01b	2.7±0.08a	1.50±0.024a	5.86±0.03b	41±2.2a	9.6±0.04a
-1.19	0.6±0.03b	2.4±0.20a	1.39±0.003a	5.42±0.03bc	38±3.2a	7.4±0.14c
Mean	0.6±0.03	2.6±0.17	1.50±0.015	6.35±0.63	36±3.6	9.0±0.68
Sandy loam						
-0.01	0.6±0.03b	2.6±0.17a	1.55±0.007a	4.94±0.02c	49±0.5a	9.9±0.17a
-0.80	0.7±0.03a	2.6±0.17a	1.58±0.010a	5.82±0.32b	48±8.1a	9.0±0.13b
-1.19	0.5±0.04c	2.1±0.13a	1.42±0.012a	5.37±0.08bc	48±2.4a	6.2±0.16d
Mean	0.6±0.05	2.4±0.19	1.52±0.010	5.38±0.23	48±3.6	8.4±0.96
$LSD_{0.05}$ (SWP)	0.1^{**}	NS	0.04^{**}	0.43^{**}	NS	0.27^{**}
$LSD_{0.05}$ (Soil)	NS	NS	NS	0.35^{**}	6.2^{**}	0.22^{**}
Interaction	0.1^{**}	NS	NS	0.61^{**}	NS	0.38^{**}

SWP, soil water potential; within columns, alphabetical letters denote mean separation by $LSD_{0.05}$ for SWP by soil type interaction; **Significant at 1% level; NS, no significant difference at P=0.05.

An increase in water-deficit stress level tended, albeit inconsistently, to reduce WUE (Table 2). Significantly ($P<0.01$) low WUE was found in the most water-deficit stressed plants at -1.19 MPa on the sandy loam. Soil type did not significantly ($P>0.05$) affect WUE, as trends in WUE did not vary for plants grown on the two soil types. For the clay, the -0.01 MPa treatment had the highest WUE of 0.7. For the sandy loam, the -0.80 MPa treatment had the highest WUE, also of 0.7.

Leaf length, leaf number, plant fresh weight and dry-matter production - Spring onion leaf length was significantly ($P<0.05$) reduced with increasing soil water-deficit stress from -0.01 to -1.19 MPa (Fig. 2A). Increases in soil water-deficit generally increased numbers of green leaves, except for the -0.01 MPa treatments on the clay (Fig. 2B).

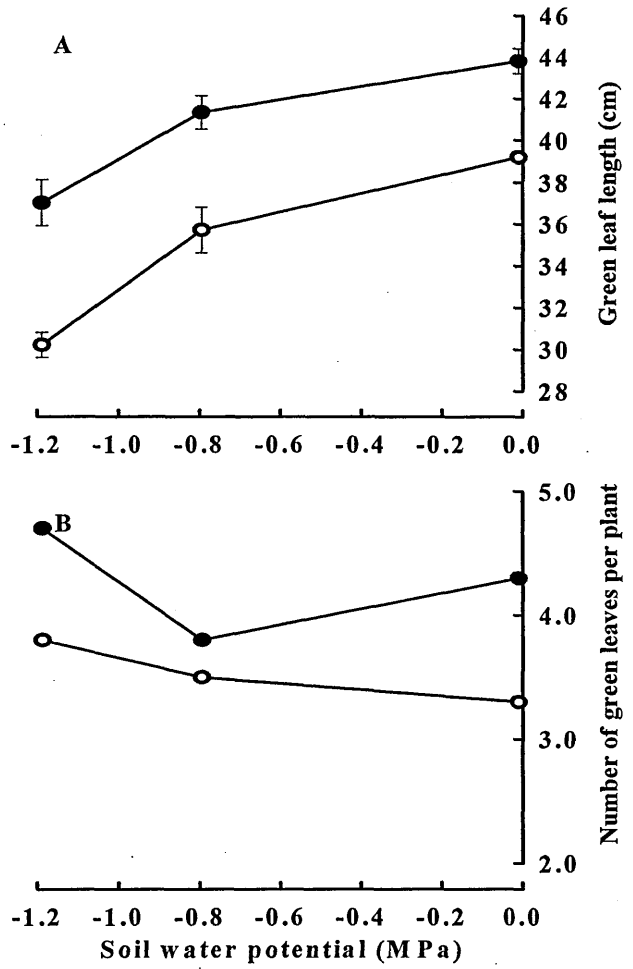

Fig. 2. Green leaf length (A) and plant height (B) of spring onion cv. White Lisbon grown on clay (filled circle) and on sandy loam (open circle) at different soil water potential levels. The vertical bars represent standard error of the means (n = 16).

Increases in green leaf number in association with reduction in leaf elongation in spring onion plants may be attributed to differential effects of water-deficit stress on cell division and cell expansion (Joyce et al., 1983) in spring onion leaves. Spring onions grown on clay produced more and longer leaves compared with plants grown on sandy loam.

Water-deficit stress effects on plant fresh weight and dry-matter production became evident 6 weeks after transplanting, and were greatest at harvest 12 weeks after transplanting (Fig. 3A-D). Reductions in LWP and RWC of water-deficit stressed plants significantly ($P<0.05$) reduced leaf elongation, plant height, fresh weight and dry-matter production. It has been reported that water stress conditions reduce leaf cell wall elasticity, and thereby inhibit leaf growth (Hsiao, 2000). This observation explains the poor growth and low dry-matter production due to large fluctuations in LWP and RWC in water-deficit stressed plants.

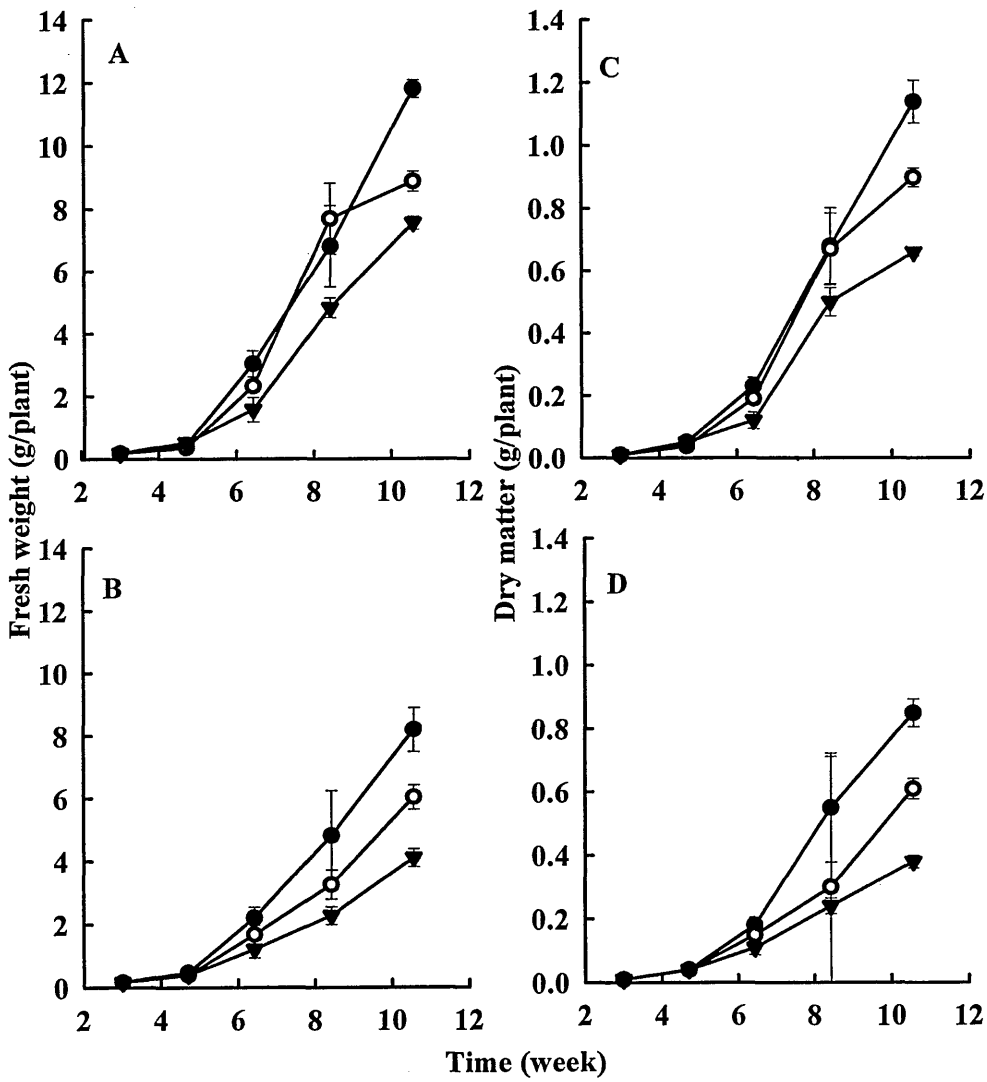

Fig. 3. Fresh weight (A, B) and dry matter production (C, D) of spring onion cv. White Lisbon grown on clay (A, C) and on sandy loam (B, D) at soil water potential levels of -0.01 (filled circle), -0.80 (open circle) and -1.19 (filled triangle) MPa. The vertical bars represent standard error of the means (n = 16).

These stress effects were most pronounced in plants subject to re-watering at -1.19 MPa. Plants grown on clay had higher fresh weight and dry-matter yield compared with sandy loam, as reported on bulb onions by Talha et al. (1978) and Mohamed et al. (1993). These responses may be attributed to inherent differences in physical and chemical properties of the clay versus the sandy loam (Table 1).

Shoot:root fresh weight ratios were neither affected by water-deficit stress nor soil type (Table 2). Transportation of nutrients and other growth factors from the roots to the shoot are related to total plant growth performance (Glenn, 2000). The balance between shoot:root fresh weight ratio suggests a strong association between total plant growth and root growth plus nutrient uptake from the rhizosphere. Water-deficit stress adversely affects physical root growth, synthesis of cytokinin by roots, and transport of other growth regulators and nutrients to the shoot (Davies et al., 1994; Glenn, 2000). Consequently, shoot growth is reduced. Therefore, the proportional change in growth maintains constant shoot:root fresh weight ratio under varying environment conditions. That the shoot:root fresh weight ratio of spring onion was not influenced by soil type and water-deficit stress levels confirms published reports (Brouwer, 1963; Forde, 2000; Hsiao, 2000).

E-nose discrimination - The 32-conducting polymer E-nose sensor measurements of %dR/R for spring onion headspace volatiles differed significantly ($P<0.05$) in response to water-deficit stress, but not soil type (Table 2). An increase in water-deficit stress on either clay or sandy loam tended, generally, to reduce %dR/R. The interaction of water-deficit stress by soil type for %dR/R was not significant ($P>0.05$).

Headspace volatile fingerprints of the spring onions are represented in the 2D PCA plot of Fig. 4. The principal components, PC 1 (X co-ordinate) and PC 2 (Y co-ordinate), together accounted for >80% of the total variance in the whole data set. PC 1 (eigenvalue = 0.179) accounted for an average of 53.4% of the total variance, while PC 2 (eigenvalue = 0.097) accounted for an average of 29.1% across soil types. Mahalanobis distance (D^2) statistics showed significant ($D^2>3.0$) separations between 2D PCA plot data set clusters for water-deficit stress level, soil type and the interaction of water-deficit stress by soil type. There were overlaps ($D^2<3.0$) of clusters of data sets on the X-Y plane, which together explained most of the total

variance.

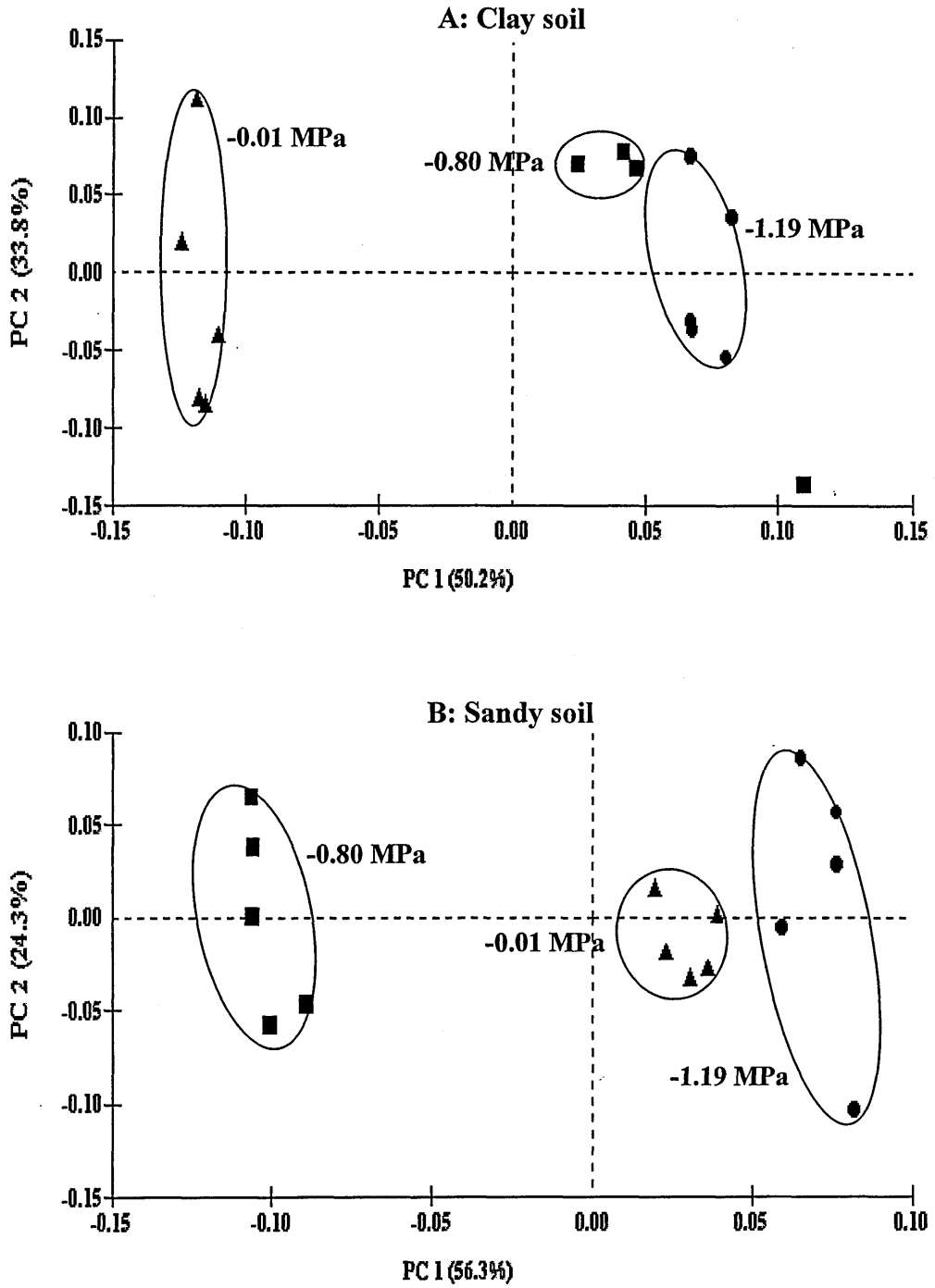

Fig. 4. 2D Principal Component Analysis plot for E-nose classification of spring onion cv. White Lisbon grown on clay (Upper panel) and on sandy loam (lower panel) at three different soil water potentials of -0.01, -0.80, and -1.19 MPa (n = 5).

The relative classification of the data sets on the 2D PCA map for the clay (upper panel) was different from that for the sandy loam (lower panel). For the clay, the data set for spring onions subject to -0.01 MPa was located to the left of the data sets for -0.80 MPa (D^2 = 20.6) and -1.19 MPa (D^2 = 59.2). For the sandy loam, the data set for plants subject to -0.80 MPa was located to the left of -0.01 MPa (D^2 = 39.2) and -1.19 MPa (D^2 = 49.7). The data sets for spring onions grown on the clay at -0.01 MPa versus plants grown on the sandy loam at -0.80 (D^2 = 2.2), and for plants grown on the clay at -0.80 MPa versus plants grown on the sandy loam at -1.19 MPa (D^2 = 0.4) were closest. Increases in water-deficit stress level reduced separations between data set clusters for spring onions grown on the clay versus on the sandy loam viz.; -0.01 MPa (D^2 = 43.2), -0.80 MPa (D^2 = 26.8) and -1.19 MPa (D^2 = 6.2).

Pyruvic acid content and lachrymatory potency - Regular watering to -0.01 MPa markedly increased pyruvic acid content of plants grown on clay by 33% as compared with spring onion plants subject to -0.80 MPa on clay. In contrast, pyruvic acid content was reduced by 15% in plants subject to -0.01 MPa on the sandy loam as compared with -0.80 MPa. Soil water-deficit stress affects both water and nutrient balance of plants (Glenn, 2000), which in turn affect the physiology and various biochemical pathways (Hsiao, 2000); such as the assimilation of S and N required for the synthesis of onion flavour compounds (Block, 1992). Moreover, water deficit adversely affects the synthesis and translocation of assimilates (Hsiao, 2000). Accordingly, water deficit can explain the significant ($P<0.01$) reductions in pyruvic acid contents by 25% at -0.80 MPa and by 30% at -1.19 MPa on the clay. Pyruvic acid content of spring onions grown on the sandy loam was inconsistent. Thus, further work is required to account for the significantly ($P<0.01$) low pyruvic acid content for regularly watered -0.01 MPa treated spring onions on the sandy loam. Overall, the pyruvic acid content in plants grown on the clay soil was slightly higher than those grown on the sandy loam (Table 2). The difference was only large for plants grown on clay at -0.01 MPa, hence the significant ($P<0.05$) interaction.

Unlike total flavour (taste and aroma) measured by pyruvic acid concentration, lachrymatory potency (pungency) was not significantly ($P>0.05$) affected by soil water level, and the interaction of water status by soil type was also not significant ($P>0.05$)

(Table 2). However, spring onions grown on the clay had significantly ($P<0.05$) higher lachrymatory potency than those grown on the sandy loam.

Total soluble solids content - Regular watering to -0.01 MPa SWP resulted in similar TSS contents for spring onions grown on both clay and sandy loam. This observation conformed to those of Mohamed et al. (1993) and Hamilton et al. (1998), who reported no significant ($P>0.05$) differences in TSS for onion bulbs grown either on clay or on sandy loam under regular irrigation. But water-deficit of -0.80 or -1.19 MPa significantly ($P<0.01$) reduced TSS irrespective of soil type, possibly due to a reduction in nutrient supply and assimilates accumulation (Hsiao, 2000). Overall, TSS of plants grown on clay was significantly ($P<0.05$) higher than for sandy loam, which can be ascribed to differences in soil physical and chemical properties (Table 2).

In conclusion, variations in irrigation regime and soil type affected tissue water status, growth, headspace volatiles and flavour of spring onion cv. White Lisbon. Reductions in growth, dry-matter content and flavour due to water-deficit stress can be explained by limitations to metabolic processes. The clay soil, by virtue of more favourable physical and chemical properties, improved growth and dry-matter production compared to the sandy loam. Clay soils have greater water storage capacity than sandy soils, and are likely to be best for spring onions when irrigation is limited and/or evapotranspiration is high. On a sandy loam it is important to supply irrigation to near field capacity in order to achieve optimum growth and dry-matter production. The influence of water-deficit stress on spring onion headspace volatiles for each soil type was shown by the different spatial arrangements of treatment-specific E-nose data set clusters on the 2D PCA map. However, the response of the sensors did not strongly correlate with the conventional measure of flavour as pyruvic acid. Further work is, therefore, required to identify chemicals in the headspace volatiles of the spring onions that interact with the sensors.

ACKNOWLEDGEMENT

The authors appreciate the technical assistance of Mr. Allen Hilton.

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CHAPTER 5: BULB ONION EXPERIMENT

5.1 Electronic nose evaluation of onion headspace volatiles and bulb quality as affected by nitrogen, sulphur and soil type

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(Submitted to *Annals of Applied Biology*, May 2003)

Summary

Edaphic factors affect the quality of onions (*Allium cepa* L.). Two experiments were carried out in the field and glasshouse to investigate the effects of N (field: 0, 120 kg ha^{-1}; glasshouse: 0, 108 kg ha^{-1}), S (field: 0, 20 kg ha^{-1}; glasshouse: 0, 4.35 kg ha^{-1}) and soil type (clay, sandy loam) on onion quality. A conducting polymer sensor electronic nose (E-nose) was used to classify onion headspace volatiles. Relative changes in the E-nose sensor resistance ratio (%dR/R) were reduced following N and S fertilisation. A 2D Principal Component Analysis (PCA) of the E-nose data sets accounted for ca 100% of the variations in onion headspace volatiles in both experiments. For the field experiment, E-nose data set clusters for headspace volatiles for no N added onions overlapped (D^2=1.0) irrespective of S treatment. Headspace volatiles of N-fertilised onions for the glasshouse sandy loam also overlapped (D^2=1.1) irrespective of S treatment as compared with distinct separations among clusters for the clay soil. N fertilisation significantly ($P<0.01$) reduced onion bulb pyruvic acid concentration (flavour) in both experiments. S fertilisation increased pyruvic acid concentration significantly ($P<0.01$) in the glasshouse experiment, especially for the clay soil, but had no effect on pyruvic acid concentration in the field. N and S fertilisation significantly ($P<0.01$) increased lachrymatory potency (pungency), but reduced total soluble solids (TSS) content

in the field experiment. In the glasshouse experiment, N and S had no effect on TSS. TSS content was increased on the clay by 1.2-fold as compared with the sandy loam. Onion tissue N:water-soluble SO_4^{2-} ratios of between 5 and 8 were associated with greater %dR/R and pyruvic acid concentration values. N did not affect inner bulb tissue microbial load. In contrast, S fertilisation reduced inner bulb tissue microbial load by 80% in the field experiment and between 27% (sandy loam) and 92% (clay) in the glasshouse experiment. Overall, onion bulb quality discriminated by the E-nose responded to N, S and soil type treatments and reflected their interactions.

Key words: onion, electronic nose, headspace volatiles, nitrogen, sulphur, soil type

Introduction

Increased consumer demand for onions (*Allium cepa* L.) generated a rise in total world production from 3,625 x 10^3 MT in 1997 to 4,184 x 10^3 MT in 2002 (FAOSTAT, 2002). Onion yield and quality attributes such as bulb size and dry-matter content, shape, colour, aroma and taste are important marketing features (Rubartzky & Yamaguchi, 1997). Yield and quality attributes are cultivar-dependent, but also vary according to environmental variables and management practices (Randle, 1997).

Onion flavour precursors, collectively termed alken(yl)-L-cysteine sulphoxides (ACSO), are found in the cytoplasm of the cells comprising intact tissues (Lancaster & Kelly, 1983; Block, 1992; Bacon *et al.*, 1999). The ACSO 1-propenyl cysteine sulphoxide (1-PRENCSO) is the most important, and is associated with the sensory attributes perceived during processing or eating (Schwimmer, 1967). Other ACSOs found in smaller amounts include methyl cysteine sulphoxide (MCSO) followed in concentration by propyl cysteine sulphoxide (PCSO). Following onion tissue disruption, the enzyme cysteine sulphoxide lyase (alliinase) is released from the vacuole and catabolises ACSOs (Lancaster & Kelly, 1983; Bacon *et al.*, 1999). The end products of this catabolism include sulphenic acid (thiosulphinate), pyruvic acid, ammonia and various other volatiles and non-volatile sulphur compounds. The type of thiosulphinate produced is specific to each flavour precursor compound hydrolysed (Randle *et al.*, 1994). Thiosulphinates and their derivatives such as thiopropanal sulphoxide, a tear stimulant formed only from 1-PRENCSO, give rise to the

characteristic onion flavour and pungency (Freeman & Whenham, 1975; Block, 1992; Bacon *et al.*, 1999).

Gas chromatography-mass spectroscopy (GC-MS) analysis of onion identified 1-propanesulphinothioic acid S-(E, Z)-1-propenyl ester ($C_6H_{12}S_2O$), (E)-1-propenesulphinothioic acid S-methyl ester ($C_4H_8S_2O$), methanesulphinothioic acid S-(E, Z)-1-propenyl ester ($C_4H_8S_2O$), 1-propanesulphinothioic acid S-1-propyl ester ($C_6H_{14}S_2O$), methanesulphinothioic acid S-1-propyl ester ($C_4H_{10}S_2O$), 1-propanesulphinothioic acid S-methyl ester ($C_4H_{10}S_2O$), methanesulphinothioic acid S-methyl ester ($C_2H_6S_2O$) and cis/trans zwiebelanes [-2,3-dimethyl-5,6-dithiabicyclo[EC 2.1.1]hexane 5-oxide ($C_6H_{10}S_2O$) in the headspace volatiles (Block *et al.*, 1992; Randle *et al.*, 1994). These organo-sulphur compounds contribute to the total flavour of onions and other Alliums.

Nitrogen (N) or sulphur (S) fertilisation affects the relative proportions of thiosulphinates and other flavour components of onions (Randle *et al.*, 1994; Randle, 2000). The interaction effect of N and S on onion flavour content is not so well documented. Increased N fertiliser application from 0.22 to 0.60 g l^{-1} increased total ACSO concentration from 1.90 to 3.67 mg g^{-1} fresh weight (fw) (Randle, 2000). The increase in ACSO concentration was due to increases in the proportions of MCSO, PCSO, and to a lesser extent 1-PRENCSO by 2.5-, 2.1- and 1.1-fold, respectively. Onion flavour, measured in terms of enzymatically produced pyruvic acid concentration, increased by 18% upon increasing N fertiliser rate from 0.22 to 0.60 g l^{-1} (Randle, 2000). Variations in the N fertiliser application rate did not affect soluble sugar concentration (Randle, 2000) and sensory panellists' perceptions of pungency, sweetness or preference (Smittle, 1984) for onion bulbs.

The importance of S nutrition in onion flavour biochemistry has been reported by Freeman & Mossadeghi (1970), Randle (1992), Randle *et al.* (1994; 1995), Randle (1997), and Hamilton *et al.* (1997; 1998). An increase in S fertiliser rate from 0.48 (15.36 mg l^{-1}) to 3.10 meq S l^{-1} (99.20 mg l^{-1}) increased thiosulphinate concentration and other related flavour compounds excluding thiopropanal sulphoxide, by 7.2-fold in onion cv. Southport White Globe (Randle *et al.*, 1994). A study on six onion clones showed that the high S rate at 7.7 meq l^{-1} (246.40 mg l^{-1}) increased enzymatically produced pyruvic acid concentration from 1.9 µmole g^{-1} fw at 0.1 meq l^{-1} to 5.5 µmole g^{-1} fw (Hamilton *et al.*, 1997). Similarly, Randle & Bussard (1993a) found for 16 onion cultivars that an increase in S fertiliser rate from 0.1 to 4.0 meq l^{-1} increased

enzymatically produced pyruvic acid concentration from 2.8 to 4.6 μmole g^{-1} fw. However, S fertilisation did not affect intrinsic (background) pyruvic acid concentration or dry-matter content (Randle & Bussard, 1993a). The carbohydrates sucrose, fructose, glucose, fructan, and other soluble sugars were not affected by S increments up to 4.4 meq l^{-1} (140.8 mg l^{-1}; Randle & Bussard, 1993a). However, Hamilton *et al.* (1997) reported a slight reduction in total sugar content in onion bulbs as S was increased from 0.1 to 7.7 meq l^{-1}.

In addition to organic (e.g. cysteine) and inorganic (e.g. SO_4^{2-}) S, elemental S can be found in plant tissues (Block, 1992; Williams *et al.*, 2002). Elemental S from gluthathione or cysteine degradation may play a role in protection of plants against disease causing pathogens (Williams *et al.*, 2002). Volatile organo-sulphur compounds derived from degradation of ACSO in Alliums also have anti-microbial properties *in vivo* (Block, 1992; Kyung *et al.*, 2002). For example, the two main anti-microbial compounds of garlic are methyl methane thiosulphinate and allyl 2-propene thiosulphinate (allicin) from methyl- and allyl- derivatives of ACSO (Kyung *et al.*, 2002). Both N and S fertilisation increase thiosulphinate levels (Randle *et al.*, 1994; Randle, 2000) and, thus, can reduce microbial load in onions. Similar effects have been reported for garlic by Kyung *et al.* (2002).

The N:S ratio of bulb tissue varied between 3 and 5 in adequately fertilised onion plants from fields in the Columbia Basin, USA (Sullivan *et al*, 2001). In other work, an N:S ratio of 15 was found to improve wheat grain cysteine concentration as compared with ratios well below or above 15 (Byers & Bolton, 1979). Cysteine is synthesised from N and S in the chloroplast before entering the glutathione cycle (Reuveny *et al.*, 1980). Cysteine then undergoes stepwise carboxylation in the glutathione cycle to produce onion flavour precursor compounds (Block, 1992). Thus, variations in N:S ratio in onions can potentially differentially affect onion flavour.

High cation exchange capacity (CEC) suggests greater soil nutrient availability for plant use (Brady & Weil, 1996; Rowell, 1996). Typically, CEC for clays range between 4 and 60 mole kg^{-1} and for sandy loam between 2 and 12 mole kg^{-1} (Rowell, 1996). Thus, plant growth and/or quality can be greater on clay soils due to high CEC than on sand soils, as has been reported for onion (Talha *et al.*, 1978) and garlic (Hanson *et al.*, 2003).

Sensory and analytical tests are conventionally used for evaluation of onion

flavour. However, panellists are subject to fatigue and inconsistency (D O'Connor, personal communication), which can render results unreliable (Giese, 2000). Analytical instruments such as high performance liquid chromatography (HPLC), gas chromatography (GC), GC-MS and spectrophotometry can quantitatively determine individual components of onion headspace volatiles (Block, 1992; Block *et al.*, 1992; Randle *et al.*, 1994; Bacon *et al.*, 1999; Yoo & Pike, 1999). Nonetheless, like sensory test, analytical determination of flavour is time-consuming, costly and need long period of development and/or training.

Relatively novel electronic nose (E-nose) sensors are comprised of either a single or an array of miniature sensors for semi-quantitative assessment of volatiles. The E-nose is considered to be reliable, fast and easy to use (Payne, 1998). Changes in electric signals resulting from the interaction of conducting polymer E-nose sensor devices with headspace volatile molecules can be used to fingerprint samples (Gopel *et al.*, 1998; Ziegler *et al.*, 1998). Pattern recognition methods, such as Principal Component Analysis (PCA) and Cluster Analysis (CA), are used to process and interpret complex signals into interpretable data (Gardner & Bartlett, 1999). Principal Components (PCs) are not correlated to each other (Wold *et al.*, 1987). Rather, each PC accounts for a proportion of the total variability in the E-nose data set. E-noses have been used to distinguish among genotypes, maturity and storage ability of some fresh produce due to their volatiles production patterns (Benady *et al.*, 1995; Persaud & Talou, 1996; Giese, 2000; Sinesio *et al.*, 2000; Keshri *et al.*, 2003). Recently, an E-nose was shown to discriminate between genetically distinct *Allium* types (Abbey *et al.*, 2001), and spring onions affected by soil type and water-deficit stress (Abbey *et al.*, 2003).

The supply and/or interaction of N and S fertilisers can have marked effects on onion growth and yield (Sachdev *et al.*, 1991; Singh *et al.*, 1996). Onions are grown on varied soils ranging from light sand to heavy clay soils (Brewster, 1994; Rubatzky & Yamaguchi, 1997). However, the effects of N and S and of soils with varying physicochemical characteristics and their interactions on onion quality, flavour and headspace volatiles are not widely reported. Bulb onion cv. Sprinters is a relatively new commercial variety in the UK. E-noses have been used to evaluate some fresh horticultural produce, but not bulb onions. This work investigates the use of a conducting polymer sensor E-nose for discrimination among headspace volatiles of onion cv. Sprinters bulbs grown on a clay and on a sandy loam either with or without

applied N and/or S. Pyruvic acid concentration, total soluble solids content and microbial load in the inner bulb tissue were also evaluated.

Materials and Methods

Field and glasshouse experiments were conducted at Cranfield University in Silsoe (latitude 52° 4 N, longitude 0° 28 W), UK between May (spring) and December (winter). Seeds of onion cv. Sprinters were obtained from Elsoms Seeds, Spalding for the study.

N and S field experiment

The field experiment on sandy loam soil (Yellowish Brown earth; Bearsted series, BC_u; Hodge *et al.*, 1984) was ploughed and harrowed in May (spring). The onion seeds were drilled at 20 cm between rows. Seedlings were thinned by hand 2 weeks after emergence to a spacing of 10 cm within rows. Fertiliser treatments were 0 (-N) or 120 (+N) kg N ha^{-1} and 0 (-S) or 20 (+S) kg S ha^{-1}. Urea ($CO(NH_2)_2$) and magnesium sulphate ($MgSO_4$; epsom salt) were N and S sources, respectively. Triple super phosphate (H_3PO_4; 125 kg P ha^{-1}) and muriate of potash (KCl; 200 kg K ha^{-1}) were applied as base macronutrient fertilisers. Fertiliser application was split, with half the rate applied at each of 3 and 5 weeks after sowing. Plants were irrigated using overhead sprinklers once a week during periods when there was no rainfall in summer. Bulbs were lifted early at 20% top-fall in October (autumn) due to wet weather conditions. The experiment design was a randomised complete block factorial with four replications. The blocks were split into two N levels (main plot factor) and two S levels (sub-plot factor). Block size was 78.8 m^2 and each treatment plot size was 6.3 m^2. Spacing in between blocks and plots were 1.0 and 0.5 m, respectively. These spaces were occupied by guard rows 0.5 m wide. Each plot was comprised of three duplicate beds.

N, S and soil types glasshouse pot experiment

Pre-germinated seeds of onion cv. Sprinters were sown into 12-cm diameter plastic pots in a glasshouse in May (spring). The pots were filled with either 500 g clay (Alluvial gley; Thames series, T_s) or 700 g sandy loam (Brown earths; Wick series, WQ_2; Hodge *et al.*, 1984) at moisture contents of 9.9% and 2.6%, respectively, on an oven-dry weight basis. Both clay and sandy loam soils were collected from the

Horticulture Research International (HRI) experimental station at Wellesbourne (UK). Each pot was thinned one week after transplanting to only one onion plant. Fertiliser treatments were 0 (-N) or 108 kg N ha^{-1} (+N; 120 mg N pot^{-1}) as urea and 0 (-S) or 4.35 kg S ha^{-1} (+S; 3.95 mg pot^{-1}) as $MgSO_4$. Basal applications of 33 kg P ha^{-1} (24 mg P pot^{-1}) as metaphosphoric acid, 64 kg K ha^{-1} (72 mg K pot^{-1}) as muriate of potash and 21 kg ha^{-1} (24 mg Mg pot^{-1}) as magnesia (MgO) were also provided in the form of nutrient solutions. N and S nutrient solutions were applied in split applications 2 and 5 weeks after transplanting. Plants were irrigated with distilled water. Soil moisture levels were maintained at $\geq$-0.01 MPa soil water potential (196.5 ml pot^{-1} for clay; 104.4 ml pot^{-1} for sandy loam). Final harvest was in December (winter) when 80% of the foliage leaves fell over on plants with applied N and S fertiliser (i.e. +N+S) over. The experiment was a split (factorial) randomised complete block design with three replications. The main plot factor was soil type, the subplot was N level and the sub-sub plot was S level. Each replication was comprised of 16 pots (plants) per treatment. Thus, the total number of plants for each treatment was 48 (i.e. 16 pots per replication by three blocks).

Data collection and analysis

E-nose evaluation

Onion bulbs were selected randomly from each treatment combination (n = 10) and homogenised in a Moulinex (Tipo 753; Patendo, Spain) mixer. The slurry was filtered after standing for 20 min to allow complete hydrolysis of ACSO by alliinase (Schwimmer & Weston, 1961). Twenty ml of filtrate was then mixed with 20 ml 5% trichloroacetic acid (TCA) in a conical flask to terminate alliinase activity. The mixture was stood for 30 min at room temperature. Equal amounts (1:1 v/v) of deionised water and the filtrate/TCA mixture were combined and vortexed. One ml of the diluted solution was put into a 100 ml Schott bottle and capped. This sample was incubated for 10 min at 25°C and 30% RH in the sample station of a 32-conduting polymer sensor E-nose (AromaScan LabStation System, model A32/8S; Osmetech Plc., UK). With the exception of the lachrymatory factor (Block *et al.*, 1992), it was anticipated that headspace volatiles would not change markedly from the time of bulb homogenisation to the time of headspace gas sampling in the E-nose. This assumption was based on the reported stability of thiosulphinates and related organo-sulphur compounds in onions as shown using GC-MS (Block *et al.*, 1992). Headspace

volatiles were sampled for a period of 70 s at a gas flow rate of 50 ml min^{-1}. The E-nose sensor unit was also set at 25°C and 30% RH. Samples runs were interspersed with sensor flushing and washing using 90% ethanol to maintain sensor consistency (AromaScan service manual). A completely randomised experiment design with eight replications was adopted. Proportional (%) changes in E-nose sensor resistance ratio (%dR/R) following interaction with sample headspace volatile molecules were recorded. %dR/R was calculated from [Ro - Rs / Ro * 100]; where Ro = base resistance and Rs = new resistance following sensor polymer interaction with sample.

Pyruvic acid analysis

Five ml of 5% TCA was added to the slurry prepared for E-nose evaluation and vortexed. After 1 h, this mixture was filtered into a 250 ml conical flask and washed with deionised water to make up 200 ml of solution mix. One ml each of 0.125% 2,4-dinitrophenylhydrazine (2,4-DNPH) and deionised water were added to 1 ml of the solution mix and vortexed. The mixture was warmed in a water bath at 37°C for 10 min. Five ml of 0.6 M NaOH was added to the warmed mixture and vortexed. A UV/VIS spectrophotometer (model PU8730; Unicam, UK) was then used to determine the absorbance at 420 nm of the onion sample extracts (Schwimmer & Weston, 1961; Randle & Bussard, 1993b). A completely randomised design with four replications was adopted.

Lachrymatory potency

The time-intensity method (Larmond, 1982; Piggott *et al.*, 1998) was used for a simple analysis of pungency (lachrymatory potency) for onion bulbs harvested from the field. Onion bulbs (n = 3) were peeled and washed in distilled water. Twenty g sub-samples of sliced bulbs from each treatment were placed in glass plates. Three digit numbers were assigned to each sample and they were randomly placed on a table. After chewing each sample, the tester's mouth was rinsed with boiled tap water. Ten min rest was allowed in between tests and 20 min after three tests. During rest, water crackers (biscuits) were eaten. The response time to maximum lachrymatory potency (hotness) during chewing was measured on a digital electronic stopwatch. The lachrymatory potency test was repeated three times for each treatment sample.

Total soluble solids content

Onion bulbs (n = 8 for each treatment) were cut and homogenised. The total soluble solids content of the homogenate was measured using a digital refractometer (model PR1; Atago Co. Ltd., Japan).

Soil and plant tissue N and water-soluble sulphate (SO_4^{2-}) analyses

Soil (n = 3) and onion plant tissue (n varied between 1 and 3 due to sometimes insufficient plant sample) N and water-soluble SO_4^{2-} concentrations were determined in the laboratory using Kjedahl and ion chromatography methods (Franklin, 1985; Rowell, 1996), respectively. For soil analysis, air-dried soils were passed through a 2-cm^2 mesh sieve before analyses of N and water-soluble SO_4^{2-} contents. Onion bulbs from the field experiment and onion bulbs plus leaf tissues from the glasshouse experiment were oven-dried at 45°C to constant weight and ground before determination of N and water-soluble SO_4^{2-} contents.

Microbial load

Onion bulbs (n = 3) were washed in sterilised distilled water and peeled. A 20 g longitudinal wedge section was cut from each bulb and homogenised in a Waring blender (Waring Commercial Blender, USA) for 30 s at room temperature. A 20 ml portion of the homogenate was then added to 180 ml sterilised 0.1% peptone water (Blanchard *et al.*, 1996) and vortexed. Three-fold dilutions were made in sterilised 0.1% peptone water before inoculation of 1 ml l^{-1} aliquots onto standard plate count agar (23.5 g l^{-1}; Apha, Oxoid) in Petri plates (8.5 cm diameter). Incubation was at 28°C for 48 h. Then the total number of colony forming units (CFU) was recorded. Each treatment had three replications.

Statistical analyses

The response signal data of the E-nose sensor to sample headspace volatiles were processed and analysed using A32/8S Microsoft Windows Version 3.24B software (AromaScan Plc., UK). The software was used to plot two-dimension (2D) Principal Component Analysis (PCA) maps. Eigenvalues (variance) were calculated by dividing the fraction of explained variance by the number of variables for each of the two axes of Principal Component one (PC 1, X-axis) and Principal Component two (PC 2, Y-axis; Wold *et al.*, 1987). Separations between centres of E-nose data set

clusters for headspace volatiles in the PCA map were determined by the Mahalanobis distance (D^2) statistic (Mahalanobis, 1941) using A32/8S software. A D^2>3.0 (i.e. three standard deviations) indicated significant separation between two clusters (Mark & Tunnell, 1985). Analysis of variance was performed using GenStat for Windows Version 4.21 (Rothamsted Experimental Station, UK) for square-root transformed %dR/R data (Gomez & Gomez, 1984), pyruvic acid, lachrymatory potency, total soluble solids content and number of colony forming units for microbial load after log transformation (Gomez & Gomez, 1984). The Least Significant Difference (LSD) method at P = 0.05 was used to determine differences between treatment means (Gomez & Gomez, 1984). Due to insufficient bulbs, especially for the glasshouse experiment, some of the treatment samples for onion tissue N and water-soluble SO_4^{2-} concentrations could not be replicated. Thus, statistical analyses for tissue N and water-soluble SO_4^{2-} concentrations were not performed. Variations in pyruvic acid concentration, lachrymatory potency and total soluble solids content were accounted for in multiple linear regression equations, MLR ($Y = \alpha + \beta_1 X_1 + \beta_1 X_2$; X_1; where Y = quality parameter, X_1 = PC 1 and X_2 = PC 2) using Minitab for Windows Version 12.13 software (Minitab Inc., USA). Both PC 1 and PC 2 were used as predictors in the MLR equation.

Results

The N and water-soluble SO_4^{2-} concentrations of the soil before the field experiment were 11.8 (±1.2) mg and 15.2 (±3.1) mg kg^{-1} at 0 to 40 cm depth. The N concentrations for the clay and the sandy loam in the glasshouse pot experiment were 687 (±23) and 316 (±14) mg kg^{-1}, respectively. The water-soluble SO_4^{2-} concentrations were 62.6 (±5.9) for the clay and 40.6 (±6.1) mg kg^{-1} for the sandy loam.

N and S field experiment

Proportional changes in the E-nose conducting polymer sensor resistance ratio %dR/R due to variations in fertiliser application ranged between 1.43 and 2.05 (Table 1). %dR/R was reduced significantly (P<0.01) by N and/or S fertilisation of onion cv. Sprinters. The interaction of N x S was significant (P<0.01). Headspace volatiles of bulbs harvested from the +N-S treatment plots gave the greatest %dR/R value followed by the -N-S treatment. The smallest %dR/R value was recorded for +N+S.

The two-dimension PCA map shows 99.9% of the E-nose data set in discrimination

of headspace volatiles of onion cv. Sprinters treated with or without N and/or S (Fig. 1). The first Principal Component PC 1 accounted for 99.7% (Eigeinvalue = 0.249) of the total variance.

Table 1. *Effects of N and S fertilisation on changes in E-nose sensor resistance ratio (%dR/R), pyruvic acid concentration, lachrymatory potency and total soluble solids content of onion bulb cv. Sprinters grown in the field.*

N rate (kg ha^{-1})	S rate (kg ha^{-1})		
	0	20	N mean
		%dR/R	
0	1.90	1.85	1.87
120	2.05	1.43	1.74
S mean	1.98	1.64	
$LSD_{(0.05)}$ N^{**}, S^{*} = 0.06 (n = 16); N x S^{**} = 0.13 (n = 8)			
		Pyruvic acid concentration (µmole g^{-1} fresh weight)	
0	13.08	11.89	12.49
120	11.24	12.52	11.88
S mean	12.16	12.21	
$LSD_{(0.05)}$ N^{**}, S^{ns} = 0.18 (n = 8); N x S^{**} = 0.25 (n = 4)			
		Time-intensity of lachrymatory potency (s)	
0	28	15	22
120	17	12	15
S mean	23	14	
$LSD_{(0.05)}$ N^{**}, S^{**} = 1.9 (n = 8); N x S^{**} = 2.7 (n = 4)			
		Total soluble solids content (%)	
0	12.6	10.3	11.5
120	9.8	10.1	10.0
S mean	11.2	10.2	
$LSD_{(0.05)}$ N^{**}, S^{**} = 0.60 (n = 8); N x S^{**} = 0.85 (n = 4)			

*, **Significant at P<0.05 and P<0.01, respectively; ^{ns}Not significantly different at P>0.05; n, number of observations used to calculate $LSD_{(0.05)}$.

The second Principal Component PC 2 accounted for only 0.2% of the total variance (Eigenvalue = 0.0005). The Mahalanobis distance (D^2) values indicated significant

(D^2>3.0) separations among the E-nose data set clusters for headspace volatiles in the PCA map with the exception of -N+S versus -N-S (D^2 = 1.0) treatments, which overlapped (Fig. 1; Table 2). The greatest variation in headspace volatiles was between data set clusters for +N+S versus +N-S followed by +N+S versus -N+S.

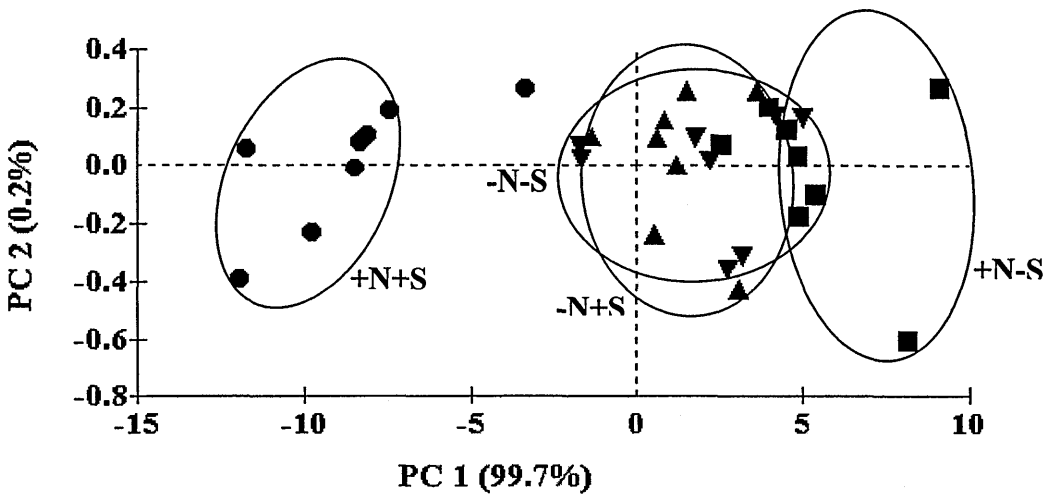

Fig. 1. Two-dimensional Principal Component Analysis (PCA) plot for E-nose data set clusters of headspace volatiles of onion cv. Sprinters affected by N and S in a field experiment (n = 8).

Table 2. *Mahalanobis distance (D^2) values for headspace volatile clusters of onion bulb cv. Sprinters affected by N and S fertilisation (n = 8) in the filed.*

	+N+S	+N-S	-N+S	+N+S	+N-S	-N+S
Field experiment						
+N-S	15.6					
-N+S	12.7	5.9				
-N-S	11.8	4.0	1.0			
Glasshouse experiment: *Clay soil*				*Sandy loam*		
+N-S	8.0			1.1		
-N+S	10.0	3.5		10.7	12.5	
-N-S	17.9	12.4	7.2	7.2	7.6	14.1

N fertilisation reduced the flavour component (pyruvic acid concentration) significantly ($P<0.01$; Table 1). In contrast, S fertilisation did not significantly ($P>0.05$) change pyruvic acid concentration of the field-grown bulbs. The N x S interaction was significant ($P<0.01$). The greatest pyruvic acid concentration was for the -N-S treatment followed by +N+S. Fertiliser treatments of -N+S and +N-S significantly ($P<0.01$) reduced pyruvic acid concentration by 9% and 14%, respectively, compared to the -N-S treatment. The response time to the measure of pungency (maximum lachrymatory potency) for onion bulb cv. Sprinters was significantly ($P<0.01$) reduced from 22 s to 15 s by N fertilisation and from 23 s to 14 s by S fertilisation (Table 1). Application of N (+N-S) or S (-N+S) alone resulted in intermediate pungency levels as compared with +N+S (very pungent) and -N-S (less pungent) treatments.

Overall, N and S fertilisation reduced bulb TSS content by 11% and 8%, respectively, compared to the -N-S treatment, which significantly ($P<0.01$) increased TSS content (Table 1). The TSS contents for onion bulbs harvested from -N+S, +N-S and +N+S treatment plots did not vary.

The highest bulb tissue N and water-soluble SO_4^{2-} concentrations were found in onions harvested from the +N-S treatment followed in magnitude of concentration by the +N+S treatment (Table 3). The lowest bulb N and water-soluble SO_4^{2-} concentration were recorded for the -N-S treatment.

N fertilisation did not significantly ($P>0.05$) affect the inner bulb microbial load (Table 3). However, S fertilisation reduced microbial load significantly ($P<0.01$) by 1.8-fold as compared with the no added S treatment. The interaction between N and S was significant ($P<0.05$). Fertiliser treatments of -N+S and +N+S significantly ($P<0.05$) reduced the internal microbial load as compared with the +N-S and -N-S treatments. The -N-S treatment gave the highest internal bulb microbial load.

Table 3. *Effects of N and S fertilisation on bulb N and water-soluble SO_4^{2-} concentrations and microbial load from inner tissue of onion cv. Sprinters grown in the field.*

N rate (kg ha^{-1})	S rate (kg ha^{-1})		
	0	20	N mean
	Bulb N concentration (g kg^{-1})		
0	7.5	8.4	8.0
120	11.5	9.2	10.4
S mean	9.5	8.8	
	Bulb water-soluble SO_4^{2-} concentration (g kg^{-1})		
0	0.94	1.04	0.99
120	2.15	1.83	1.99
S mean	1.55	1.44	
	Microbial plate count (x10^3 CFU g^{-1})		
0	14.7	1.7	8.2
120	4.6	2.0	3.3
S mean	9.7	1.9	
$LSD_{(0.05)}$	N^{ns}, S^{**} = 0.41 (n = 6); N x S^{*} = 0.57 (n = 3)		

CFU, colony forming unit; *, **Significant at P<0.05 and P<0.01, respectively; ^{ns}not significant at P>0.05; n, number of observations used to calculate $LSD_{(0.05)}$.

N, S and soil type glasshouse pot experiment

N fertiliser alone had no significant (P>0.05) effect on %dR/R compared to the significant (P<0.01) effects of S fertiliser alone and soil type in the glasshouse experiment (Table 4). The only significant (P<0.05) interaction effect was N fertiliser by soil type. The greatest %dR/R value was recorded for headspace volatiles of N-fertilised onion cv. Sprinters grown on the sandy loam. In contrast, onions fertilised with both N and S on the clay soil gave the smallest %dR/R value. The S fertiliser treatment and the clay soil significantly (P<0.01) reduced %dR/R as compared with the N fertilisation and the sandy loam.

The 2D PCA plots for the clay (Fig 2A) and the sandy loam (Fig. 2B) treatments showed that PC 1 alone accounted for over 99.7% of the variations in E-nose data set clusters for headspace volatiles of onion cv. Sprinters. The explained variance for PC 1 represented an Eigenvalue of 0.249. PC 2 explained only a small fraction of 0.1%, which represented an Eigenvalue of 0.0003. There were significant (D^2>3.0)

separations among E-nose data set clusters due to treatment effects on headspace volatiles (Fig. 2; Table 2). For the clay, the greatest separation of E-nose data set clusters for headspace volatile was between the +N+S versus the -N-S treatments.

Table 4. *Effects of N, S and soil type on changes in E-nose sensor resistance ratio (%dR/R), pyruvic acid concentration and total soluble solids content of bulb onion cv. Sprinters grown in the glasshouse.*

N rate	Clay			Sand		
(kg ha^{-1})	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
	0	4.35	N mean	0	4.35	N mean
			%dR/R			
0	1.87	1.67	1.77	1.69	1.45	1.57
108	1.53	1.22	1.37	1.90	1.85	1.88
S mean	1.70	1.45	1.57^{C}	1.80	1.65	1.73^{S}
$LSD_{(0.05)}$	N^{ns}, S^{**}, Soil** = 0.06 (n = 32); N x S^{ns}, N x soil**, S x soil^{ns} = 0.09 (n = 16); N x S x soil^{ns} = 0.12 (n = 8)					
	Pyruvic acid concentration (µmole g^{-1} fresh weight)					
0	6.49	10.81	8.65	5.72	7.73	6.73
108	4.86	7.84	6.35	4.80	8.06	6.43
S mean	5.68	9.33	7.50^{C}	5.26	7.90	6.58^{S}
$LSD_{(0.05)}$	N^{**}, S^{**}, Soil** = 0.07 (n = 16); N x S^{ns}, N x soil**, S x soil** = 0.11 (n = 8); N x S x soil** = 0.15 (n = 4)					
	Total soluble solids content (%)					
0	10.8	11.6	11.2	8.6	9.0	8.8
108	10.9	10.3	10.6	8.9	10.0	9.4
S mean	10.9	11.0	10.9^{C}	8.8	9.5	9.1^{S}
$LSD_{(0.05)}$	N^{ns}, S^{ns}, Soil** = 0.47 (n = 16); N x S^{ns}, N x soil**, S x soil^{ns} = 0.66 (n = 8); N x S x soil* = 0.94 (n = 4)					

$^{C, S}$Soil mean for clay and sandy loam, respectively; *, **Significant at P<0.05 and P<0.01, respectively; ^{ns}Not significantly different at P>0.05; n, number of observations used to calculate $LSD_{(0.05)}$.

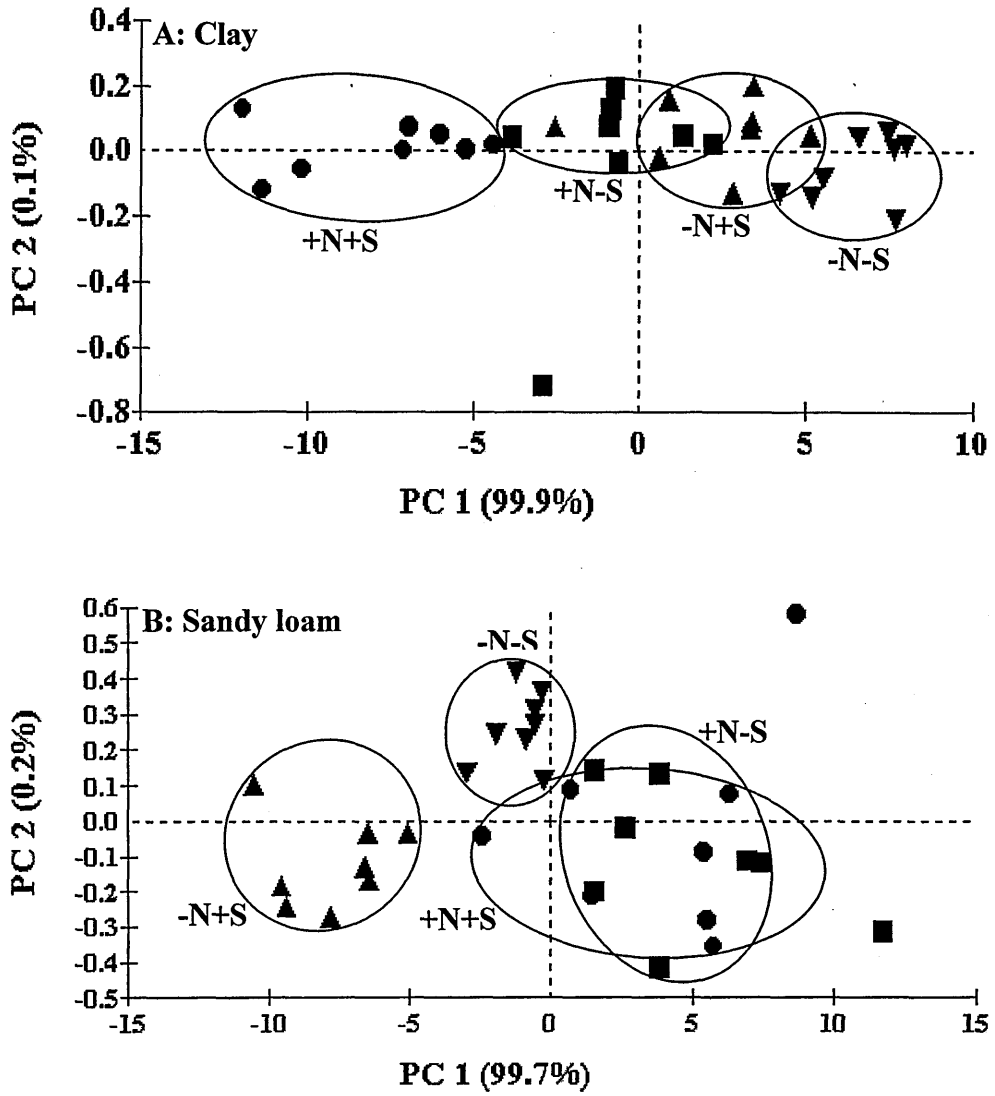

Fig. 2. Two-dimensional Principal Component Analysis (PCA) plots for E-nose data set clusters of headspace volatiles of onion cv. Sprinters affected by N and S on clay (A) and on sandy loam (B) in a glasshouse experiment (n = 8).

A small, but significant (D^2>3.0) separation was also found between the +N-S versus the -N+S treatments on the clay. For the sandy loam, clusters for the -N+S versus the -N-S treatment showed the greatest separation. The E-nose data set clusters for the +N-S versus the +N+S treatments on the sandy loam overlapped (D^2<3.0). The distance between the centres of E-nose data set clusters for -N-S versus +N+S on the sandy loam was similar to that for -N-S versus +N-S treatments.

The pyruvic acid concentration of onion bulb cv. Sprinters varied significantly (P<0.01) with the N, S and soil type treatments (Table 4). The interactions amongst these treatments were also significant (P<0.01) with only the exception of the N x S

interaction. N fertiliser reduced pyruvic acid concentration significantly ($P<0.01$).

The proportional reduction in pyruvic acid concentration due to N fertilisation was 26% on the clay and 4% on the sandy loam as compared with no N addition. In contrast, pyruvic acid concentration in S-fertilised onions was increased by 64% on the clay and by 50% on the sandy loam as compared with no S addition. Overall, the -N+S treatment on the clay and the +N+S treatment on the sandy loam gave the highest pyruvic acid concentration. The lowest pyruvic acid concentration was in bulbs harvested from the +N-S treatment plots for both clay and sandy loam soils.

Neither N nor S treatment significantly ($P>0.05$) influenced TSS content as compared with the soil type treatment, whereas the clay soil enhanced bulb TSS (Table 4). The TSS content for the -N-S treatment versus the +N-S treatment was similar for the clay and for the sandy loam.

N fertiliser application increased total plant tissue (foliage plus bulb) N concentration by 1.6-fold, but was reduced by 0.3-fold upon S fertilisation across both soil types (Table 5). Total plant tissue N concentration was greatest for the +N-S treatment on both the clay and the sandy loam. N and S fertiliser application on the clay reduced the total plant tissue water-soluble SO_4^{2-} concentration by 0.7- and 0.2-fold, respectively. In contrast, both N and S fertilisation on the sandy loam soil increased the total plant tissue water-soluble SO_4^{2-} concentration by 1.2- and 1.3-fold, respectively. Similar values of tissue water-soluble SO_4^{2-} content were found for the -N+S and the +N+S treatments on either the clay or the sandy loam.

Microbial load in the inner bulb tissue did not vary significantly ($P>0.05$) between N treatments (Table 5). The effects of S and soil type and the interactions of S and/or soil type versus N were highly significant ($P<0.01$). On average, microbial load declined by 92% on the clay and by 27% on the sandy loam as a consequence of the S treatment. The lowest microbial load was recorded for the -N+S treatment on both the clay and the sandy loam treatments. The +N-S treatment greatly increased microbial load on both soil types. Overall, microbial load was 75% less on the clay than on the sandy loam.

Table 5. *Effects of N, S and soil type on total plant N and water-soluble SO_4^{2-} concentrations and microbial load from inner tissues of bulb onion cv. Sprinters grown in the glasshouse.*

N rate	Clay			Sand		
(kg ha^{-1})	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
	0	4.35	N mean	0	4.35	N mean
	Total plant N concentration (g kg^{-1})					
0	13.7	16.9	15.3	16.1	15.0	15.6
108	30.5	14.2	22.4	36.8	18.5	27.7
S mean	22.1	15.6	18.8^{C}	26.5	16.8	21.6^{S}
	Total plant water-soluble SO_4^{2-} concentration (g kg^{-1})					
0	2.8	2.2	2.5	1.7	3.8	2.7
108	0.7	0.8	0.8	3.1	3.4	3.3
S mean	1.8	1.5	1.7^{C}	2.4	3.6	3.0^{S}
	Microbial plate count (x10^3 CFU g^{-1})					
0	120.3	7.0	63.7	268.0	235.3	251.7
108	142.0	13.7	77.8	398.7	250.0	324.3
S mean	131.2	10.4	70.8^{C}	333.4	242.7	288.0^{S}

$LSD_{(0.05)}$: N^{ns}, S^{**}, $Soil^{**}$ = 1.36 (n = 12); N x S^{**}, N x $soil^{**}$, S x $soil^{**}$ = 1.55 (n = 6); N x S x $soil^{**}$ = 1.85 (n = 3).

$^{C, S}$Soil mean for clay and sandy loam, respectively; *, **Significant at P<0.05 and P<0.01, respectively; ^{ns}not significant at P>0.05; n, number of observations used to calculate $LSD_{(0.05)}$.

Discussion

Charge flow generally increased (i.e. smaller %dR/R values) within the E-nose sensor polymers during interaction with headspace volatiles of N- and S-fertilised onions (Tables 1 and 4). This effect was moreso when both N and S were added to the field experiment sandy loam soil and the clay in pots in the glasshouse. In contrast, the N plus S fertiliser treatments on sandy loam in the glasshouse reduced charge flow (i.e. increased %dR/R). Thus, generally, a limitation in N and/or S supply differentially affected %dR/R in the field and the glasshouse experiments. The two Principal Components PC 1 (X-axis) and PC 2 (Y-axis) accounted for nearly 100% of the total variance in the E-nose data set for headspace volatiles of onion cv. Sprinters (Figs 1 and 2). Thus, the two PCs accounted for almost all the distribution of information in the E-nose data set (Wold *et al.*, 1987). Over 99% of this information

was contained in PC 1 alone. Changes in the E-nose sensor conductivity %dR/R and the variations in data set cluster locations in the 2D PCA maps can therefore be ascribed to differences in onion cv. Sprinters plant responses to the N, S and/or the soil type treatments.

Plant growth and harvest quality are affected by environmental variables and inherent soil properties such as nutrient, energy and water balance (Beverly *et al.*, 1993). For instance, amounts of N (Randle, 2000) and S (Randle *et al.*, 1994; 1995) supply were shown to differentially affect the concentrations of the onion flavour precursor compound, ACSO. Consequently, the concentrations of thiosulphinates, sulphines and zwiebelanes detected in the headspace of homogenised onions using GC-MS (Block *et al.*, 1992; Randle *et al.*, 1994) can vary. It follows that the composite effects of the N, S and/or the soil type treatments on such volatile organo-sulphur compounds influenced the E-nose sensor polymers (Bartlett *et al.*, 1997). Adsorption and desorption processes led to changes in charge flow through the sensor unit (Osmetech, 1999). Similarities in the composition of headspace volatiles were apparently reflected in overlaps of E-nose data set clusters in the PCA maps (Sinesio *et al.*, 2000; Keshri *et al.*, 2003).

The reduction in pyruvic acid concentration following applications of N fertiliser (Tables 1 and 4) concurs with a report by Randle (2000). The effect of N can be attributed to a reduction in the concentration of the major onion flavour precursor compound, 1-PRENCSO. External sources of S such as rainwater or irrigation water and greater soil volume for root exploration in the field probably masked treatment differences between field plots with versus without S fertiliser application (Tables 1 and 3) (Olson & Rehm, 1986; Hamilton *et al.*, 1998). Field-grown onions that were not supplied with either N or S gave the highest pyruvic acid concentration, but had the least lachrymatory potency (pungency; Table 1). These effects suggest that pyruvic acid concentration is independent of the lachrymatory factor, which reflects thiopropanal sulphoxide produced during hydrolysis of ACSO (Block, 1992; Bacon *et al.*, 1999). For the glasshouse experiment, however, the no fertiliser added treatment reduced bulb pyruvic acid concentration. S fertilisation in the glasshouse experiment increased the pyruvic acid concentration of onion cv. Sprinters. This effect is consistent with reports for other onion cultivars (Randle, 1992; Randle *et al.*, 1994; Hamilton, 1997; 1998). S fertilisation is generally known to increase ACSO concentration, especially 1-PRENCSO, leading to an increase in flavour as suggested

by the present findings.

The reductions in total soluble solids content in onion cv. Sprinters due to the N and S fertiliser treatments can be linked to reductions in total non-structural water-soluble carbohydrates (Tables 1 and 4). Similarly, Randle (1992) reported that these quality parameters are both reduced by S fertilisation in some onion cultivars, as was found with cv. Sprinters.

The range for bulb N (i.e. 0.75 to 1.15%) and total plant N (i.e. 1.37 to 3.68%; Tables 3 and 5) fell within documented ranges for bulbs (i.e. 0.55 to 19.3%) and foliage (i.e. 2.32 to 4.47%) (Smittle, 1984; Sachdev *et al.*, 1991; Singh *et al.*, 1996; Randle, 2000). For SO_4^{2-} concentrations in onion bulbs, Randle (2000) reported a range of 0.40 to 0.44% on dry weight basis. In the present work, the range for the proportion of water-soluble SO_4^{2-} concentration was between 0.09 to 0.22% for the bulb and 0.07 to 0.38% for the whole plant. The low N and water-soluble SO_4^{2-} concentrations in this study as compared with other reports may be due to differences in onion bulb variety and the early harvest (Lancaster *et al.*, 1986; Sullivan *et al.* 2001) in the field experiment as a result of wet weather conditions. The N:water-soluble SO_4^{2-} ratio ranged from 5 to 8 for the field experiment, 5 to 44 for the glasshouse clay and 4 to 12 for the glasshouse sandy loam. Sullivan *et al.* (2001) reported an N:S ratio of between 3 and 5 for bulb onion.

The treatments that gave the highest pyruvic acid concentration were -N-S for the field sandy loam and -N+S for the clay and +N+S for the sandy loam in pots in the glasshouse. These treatments had the second highest %dR/R values and N:water-soluble SO_4^{2-} ratios ranging between 5 and 8. N:water-soluble SO_4^{2-} ratios <5 or >8 did not show any association with pyruvic acid concentration or %dR/R. In a previous report, Randle (1992) found a low ($r = 0.30$), but highly significant ($P<0.01$) linear association for proportion (%) of total bulb S versus enzymatically produced pyruvic acid concentration.

The reduction in microbial load in the inner tissue of S-fertilised onion cv. Sprinters (Tables 3 and 5) may be attributed to increases in elemental S and organo-sulphur compounds. These are known to have anti-microbial function (Block, 1992; Kyung *et al.*, 2002; Williams *et al.*, 2002).

Combination of PC 1 and PC 2 as predictors in multiple linear regression (MLR) equations did not significantly ($P>0.05$) explain variations in pyruvic acid concentration, lachrymatory potency and total soluble solids content in either field or

glasshouse experiments (Table 6). Nonetheless, for the clay treatment the r^2 for the MLR equation for total soluble solids was significant ($P<0.05$) but small.

Table 6. *Multiple linear regression equation (MLR; $Y = \alpha + \beta_1 X_1 + \beta_1 X_2$) of pyruvic acid concentration, lachrymatory potency and total soluble solids content on Principal Components one (PC 1) and two (PC 2) for onion cv. Sprinters evaluated using an E-nose.*

Variate (Y)	MLR equation	r^2	Significance	SE
	N and S field experiment			
Pyruvic acid (μM g^{-1} fw)	$Y = 12.2^{**} - 0.06^{ns}PC\ 1 + 0.07^{ns}PC\ 2$	19%	P = 0.26	0.02
Lachrymatory potency (s)	$Y = 18.2^{**} + 0.05^{ns}PC\ 1 - 1.10^{ns}PC\ 2$	21%	P = 0.22	1.50
Total soluble solids (%)	$Y = 10.7^{**} + 0.03^{ns}PC\ 1 + 0.76^{ns}PC\ 2$	2%	P = 0.86	0.34
	N, S and soil type glasshouse experiment			
Clay				
Pyruvic acid (μM g^{-1} fw)	$Y = 7.48^{**} + 0.01^{ns}PC\ 1 + 5.69^{ns}PC\ 2$	10%	P = 0.51	0.58
Total soluble solids (%)	$Y = 10.9^{**} + 0.73^{ns}PC\ 1 + 3.54^{ns}PC\ 2$	46%	P = 0.02	0.17
Sandy loam				
Pyruvic acid (μM g^{-1} fw)	$Y = 6.58^{**} - 0.09^{ns}PC\ 1 - 1.39^{ns}PC\ 2$	16%	P = 0.32	0.35
Total soluble solids (%)	$Y = 9.09^{**} + 0.04^{ns}PC\ 1 - 0.80^{ns}PC\ 2$	16%	P = 0.31	0.15

fw, fresh weight; SE, standard error of the MLR; **Significant at $P<0.01$; ^{ns}not significant at $P>0.05$; number of observations (n) used for the MLR equation is 16.

The significance ($P<0.01$) of the constant α in the MLR equations suggests that the forms of the equations were dependent on the N and/or S treatments. Pyruvic acid concentration in field-grown onions increased with increases in lachrymatory potency ($r = 0.54$) and total soluble solids content ($r = 0.69$, $P<0.01$, $n = 16$). Also, a linear association exists between lachrymatory potency and total soluble solids ($r = 0.76$, $P<0.01$). For the glasshouse pot experiment, the r-value for pyruvic acid concentration versus total soluble solids content across soil type was 0.45 ($P<0.05$, $n = 32$). Pyruvic acid concentration ($r = -0.19$) and total soluble solids ($r = -0.01$) did not linearly associate significantly ($P>0.05$) with %dR/R.

In conclusion, this work shows that the conducting polymer sensor E-nose can discriminate onion qualities as affected by N, S and soil type treatments and their interactions. Both N and S fertilisation to onion plants on clay versus sand soil reduced the E-nose sensor polymer conductivity. The acquired E-nose data sets

accounted for nearly all the sensed variation in headspace volatiles of onion cv. Sprinters. It was found that S fertilisation increased pyruvic acid concentration and reduced the ratio of plant tissue N:water-soluble SO_4^{2-}, %dR/R and microbial load in inner bulb tissues. The conventional measures of onion quality of pyruvic acid, lachrymatory potency and total soluble solids did not correlate with %dR/R. Further work, possibly involving sensor design, is required to determine exactly which onion headspace volatile chemicals interact most strongly with the E-nose sensor polymers. Understanding the chemical compounds in the onion headspace volatiles that interacted with the sensor polymer could enable the establishment of clear correlations between onion qualities versus E-nose data sets. Similarly, additional studies might seek to establish recommendation ranges for N:S ratios versus onion quality characteristics including flavour.

Acknowledgement

The authors are grateful for technical assistance from Mr. Allen Hilton.

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5.2 Quality assessment of diced onion using an electronic nose

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(Submitted to *Journal of the Science of Food and Agriculture*, December 2002)

Abstract: Cutting of onion (*Allium cepa* L.) bulbs initiates biochemical, physiological and microbiological processes that reduce shelf life. Cut onion spoilage can be monitored by sensory and analytical methods, but these are usually costly, time-consuming and/or technically complex. Evaluation of diced onion quality using a potentially more efficient 32-conducting polymer sensor electronic nose (E-nose) was investigated. Diced (ca. 6 mm^3) brown onion was sealed in 50 μm thick polyethylene bags and stored for 3, 6 or 9 days at 4°C. E-nose sensor response (%dR/R) to samples headspace gas did not change significantly ($P>0.05$) over the initial 6 days of storage. However, %dR/R significantly ($P<0.05$) reduced from 1.91% on day 0 to 1.70% on day 9. Mahalanobis distance (D^2) values for separation of headspace volatiles data set clusters increased with later sampling times; being 3.6, 5.8 and 7.0 on days 3, 6 and 9, respectively, with reference to day 0. Pyruvic acid concentration reduced significantly ($P<0.01$) by 12, 13 and 27% on these days. Greatest reduction in dry-matter content, from 18 to 16%, was recorded between days 0 and 3. Time to maximum lachrymatory potency (hotness) increased from 34 s response time on day 0 to 42 s on day 3, and could not be sensed on days 6 and 9. Total soluble solids content did not change significantly ($P>0.05$) during 9 days of storage. A positive linear correlation was found for %dR/R (Y) versus pyruvic acid concentration (X); where, $Y = 1.166 + 0.879X$; $r = 0.803$; $P>0.05$; $n = 4$. Collectively, these results suggest that the conducting polymer sensor E-nose could be used to monitor quality of minimally processed onion.

Keywords: *Allium cepa*; E-nose sensor; minimally processed onion; pyruvic acid

INTRODUCTION

Onions (*Allium cepa* L.) are eaten for their distinctive aromas and tastes.[1] There is a trend of increasing consumer demand for minimally processed foods,[2] including fresh cut onion. Preference for minimally processed food can be attributed to produce freshness, safety, availability and convenience. However, minimally processed fresh foods have short shelf lives compared with their intact counterparts.[3,4] Thus, it is important to monitor their quality. Quality is currently evaluated mostly by sensory methods.[5,6]

The harvested onion bulb is a living organ prone to stress leading to alteration of normal metabolic activities and to senescence.[7-9] Minimal processing techniques such as dicing, wedging, slicing and ringing of onion bulbs cause tissue injury and reduce shelf life.[10-13] For example, reductions in sugar concentration and other food reserves are faster in diced onion bulbs as compared with intact bulbs.[11] Such differences can be attributed to increased rates of respiration, since there is increased oxygen influx into diced onion tissues. Rapid superficial microbial development fuelled by the release of cellular fluid during cutting processes also increases overall respiration rate and enhances discolouration of fresh cut onion.[8,10,14]

Headspace flavour volatiles for diced onion in modified atmosphere package (MAP) bags reduce in concentration with increasing duration of storage.[10] Similarly, total flavour as indicated by pyruvic acid content reduces with increasing storage period.[11] Moreover, reduction in flavour quality of minimally processed onion in MAP bags is associated with increases in off-flavour compounds, such as methanethiol and propanethiol.[15] Anaerobic conditions created as tissue senesces, and as a result of microbial activity, may also contribute off-flavour compounds; including ethanol, ethyl acetate and acetaldehyde.[16,17]

Quality assessment of foods in general, including minimally processed onion, is conventionally by sensory and analytical tests.[5,6] However, these conventional tests can be costly, time-consuming and technically complex. Moreover, results from sensory appraisals of onion samples may be neither reliable nor repeatable due to panellist fatigue and inconsistencies (O'Connor D pers comm). In recent times, relatively novel electronic nose (E-nose) technology has been used to evaluate storage qualities of tomato,[18] meat, fish, edible oils and fats[19] and milk,[20] but not onions. Cluster analysis and principal component analysis along with changes in E-nose sensor conductivity were used to discriminate odour quality.

The conducting polymer-based E-nose sensor is comprised of an array of tiny elements that interact with headspace volatile molecules. Conductance of the various individual polymer units alters upon absorption and desorption of volatiles. Resultant resistance signals are processed using mathematical algorithms to yield output data that fingerprint the odour characteristics of the sample presented.[6,21] E-nose devices are safe, easy to use and cost-effective. Relatively large numbers of samples can be analysed per unit time, and automation of odour evaluation is possible.[6,22]

This study investigates the use of a 32-conducting polymer sensor E-nose for monitoring quality changes in diced onion processed and packaged by a UK wholesale food supply company.

EXPERIMENTAL

Sample preparation

Brown onion bulbs were peeled and left for 24 hr to equilibrate to room temperature in a commercial food processing factory (Parripak Foods Ltd., UK). The peeled onion bulbs were disinfected by dipping in 5 $\mu g\ l^{-1}$ chlorine water before dicing to ca. 6 mm^3. The diced onion (250 g) was sealed in ca. 1.3 l capacity 50 μm thick transparent polyethylene film bags with an oxygen permeability of 49,500 $cm^3\ O_2\ linear^{-1}\ metre^{-1}\ day^{-1}\ atm^{-1}$ (P-Plus, Danisco Flexible Ltd., UK).

Storage and experiment design

The diced onion sealed in polyethylene film bags was stored at 4°C. A randomised complete block design was adopted with three replications. Each block (a tray) was comprised of two sample bags of sealed diced onions for each storage period of 3, 6 or 9 days. Thus, there were 6 bags arranged in a single layer on each tray. Diced onion for initial assessment (day 0) was stored under the same conditions for 20 hr before analysis. This delay was adopted because minimally processed onion from the factory reaches sales destinations within 24 hr of processing, packaging and handling at low temperature.

Data collection and statistical analyses

E-nose discrimination

Diced onion for each time treatment from each of the three blocks was homogenised

in a Moulinex (Tipo 753; Patendo, Spain) mixer at room temperature. After 20 min, this bulk homogenate was mixed (1:1 w/v) with 5% trichloroacetic acid (TCA) for 20 s to terminate alliinase activity. One ml of the resultant solution was placed in a 100 ml Schott bottle and moved into the sample station of an AromaScan LabStation System (Model A32/8S; Osmetech, UK) to equilibrate for 10 min at 25°C and 30% RH. Headspace gas was then sampled for a period of 70 s at a gas flow rate of 50 ml/min. The response of the E-nose 32-conducting polymer sensor to the headspace volatiles was processed and analysed using A32S Microsoft Windows Version 3.24B software (AromaScan Plc., UK).

Pyruvic acid concentration

One ml aliquots of homogenate/TCA (1:1 v/v) solution were dispensed into conical flasks. Then, 1 ml aliquots each of 0.0125% (w/v) 2,4-dinitrophenylhydrazine (2,4-DNPH) and deionised water were added. This cocktail was mixed for 20 s and then warmed in a water bath at 37°C for 10 min. Five ml of 0.6 N NaOH was then added and the cocktail mixed for another 20 s. An UV/VIS spectrophotometer (Model PU8730; Unicam, UK) was used to measure absorbance at 420 nm. Absorbance was converted to μmole pyruvic acid g^{-1} fresh weight using a sodium pyruvate standard calibration curve.[23,24]

Sensory appraisal of lachrymatory potency (LP)

Diced onion samples (20 g) from each block were placed on glass plates and labelled with two digit numbers. Samples were selected at random and the time to maximum lachrymatory potency (time-intensity of hotness) as sensed by the tongue during chewing was recorded with a stopwatch.[25]

Total soluble solids content (TSS)

Diced onion samples were homogenised. A hand-held refractometer (Model PR1; Atago Co. Ltd., Japan) was used to determine the total soluble solids content of the homogenate.

Dry-matter content (%DM)

Diced onion samples were weighed before and after oven drying at 65°C for 48 hr.

Dry-matter content (%) was then calculated on fresh weight basis.

Data analysis and presentation

E-nose data set clusters of headspace volatiles for the different storage periods were compared in a two-dimension (2D) principal component analysis (PCA) plot. Eigenvalues (variances) were calculated by multiplying the proportion of explained variance by the number of variables for each axis, these being principal component 1 (PC1) on the X-axis and principal component 2 (PC 2) on the Y-axis.[26] The Mahalanobis distance (D^2) statistic was used to determine the degree of significance of separation between PC 1 versus PC 2 data sets of headspace volatile clusters in the 2D PCA plot.[27] A $D^2>3.0$ (i.e. three standard deviations) between two data set clusters is considered significant separation. A dendrogram was plotted by hierarchical cluster analysis of observations using Minitab for Windows Version 12.23 software (Minitab Inc., USA) to show relationships among E-nose data set clusters. E-nose sensor response data sets determined by relative changes in sensor resistance ratio (%dR/R) were transformed by the square-root transformation rule in preparation for ANOVA[28] using Minitab. However, for convenience of interpretation, the original data for %dR/R are tabulated. ANOVAs for balanced designs were also performed on pyruvic acid concentration, lachrymatory potency, total soluble solids content and percentage dry-matter. The Least Significant Difference (LSD) method was used to separate treatment means at P= 0.05.

RESULTS AND DISCUSSION

E-nose data set clusters (PC 1 versus PC 2) for headspace volatiles of the sealed diced onion separated in space with increasing storage period (Figs 1 and 2). Overall, 76% of the total variance in headspace volatiles was explained by PC 1 and PC 2 (Fig 1). PC 1 accounted for over 61% (Eigenvalue= 0.15) of the total variance as compared with 15% (Eigenvalue= 0.04) accounted for by PC 2. The relative location of each data set cluster of headspace volatiles in the PCA map was dependent on the storage period for the diced onion. Data set clusters for headspace volatiles shifted from left to right across the PCA map as storage period was extended from day 0 (initial assessment before storage) to day 6.

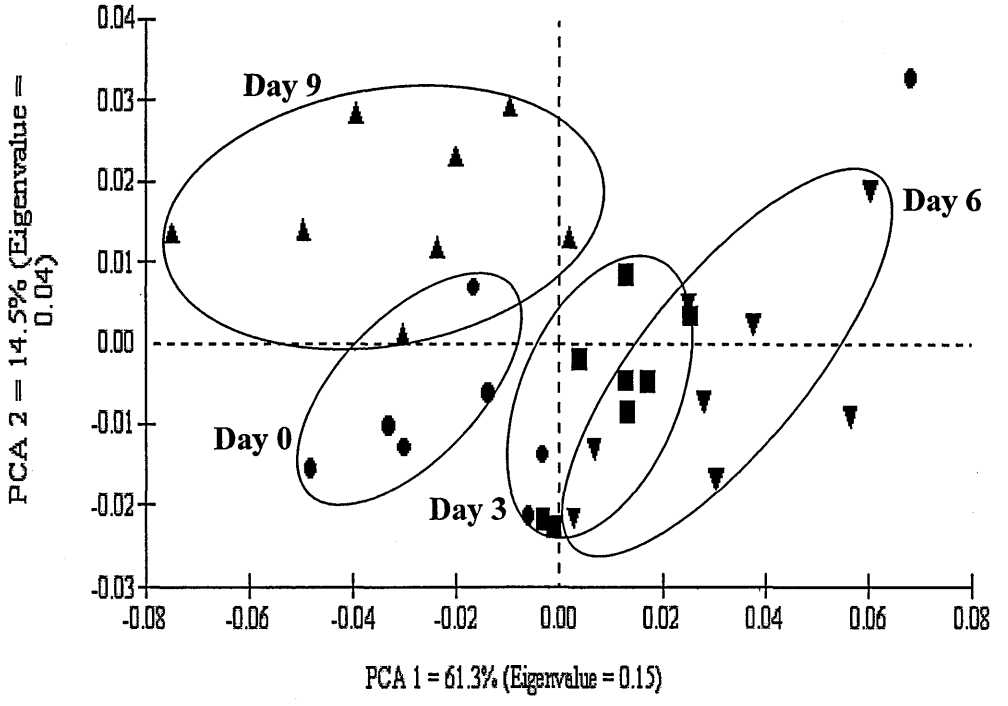

Figure 1. Two-dimensional (2D) Principal Component Analysis (PCA) plot of E-nose data set clusters for headspace volatiles of diced onion in sealed polyethylene film at three different storage periods.

The increase in storage duration resulted in significant (D^2>3.0) increases in the distances between E-nose data set clusters for each of days 3 (D^2= 3.6), 6 (D^2= 5.8) and 9 (D^2= 7.0) as compared to day 0 (Table 1). Thus, even after just 3 days there was a change in headspace volatiles for the diced onions. Interestingly, the position of data set cluster for day 9 deviated from the trend observed from day 0 up to day 6. This cluster was located up above day 0 on the left hand-side of the PCA map. The position of the data set cluster for day 9 explains the greater D^2 values for days 3 or 6 versus day 9, these being 10.1 and 11.6, respectively, as compared with 7.0 for day 0 versus day 9. The deviation from the established trend may reflect a significant change in tissue metabolism, which perhaps was due to the onset of anaerobic respiration. Further work is required to test this proposition.

Table 1. Mahalanobis distance (D^2) values for E-nose data set cluster separation on a 2D PCA plot for diced onion headspace volatiles at three different storage periods

	Day 0	Day 3	Day 6
Day 3	3.6	-	-
Day 6	5.8	4.3	-
Day 9	7.0	10.1	11.6

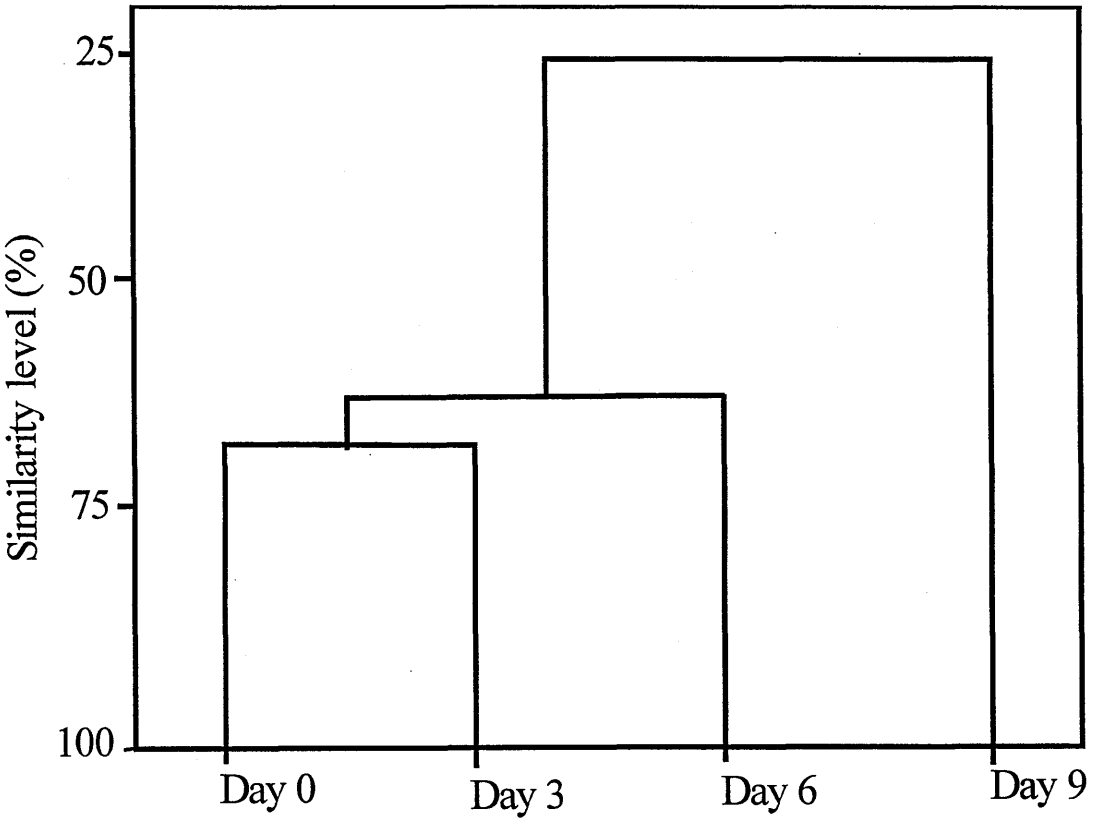

Figure 2. Dendrogram of E-nose sensor responses for headspace volatiles of diced onion in sealed polyethylene film at three different storage period.

Three different sets of E-nose data clusters for diced onion headspace volatiles were identified in the cluster analysis dendrogram (Fig 2). Cluster one consisted of an overlap of data sets for days 0 and 3, while days 6 and 9 comprised separate clusters two and three, respectively. Thus, the dendrogram suggests differences for diced onion flavour volatiles over the first 3 days of storage as compared with samples stored longer. The similarities in headspace volatiles shown in the dendrogram

support those evident in the 2D PCA plot (Figs 1 and 2).

Table 2. E-nose conducting polymer sensor resistance ratio (%dR/R), pyruvic acid content per unit fresh weight (fw), lachrymatory potency (LP), total soluble solids and dry-matter contents of diced onion in sealed polyethylene film bag at different storage period (mean±standard error)

Day	%dR/R	Pyruvic acid content (μmole g^{-1} fw)	Time-intensity of LP (s)	Total soluble solids (%)	Dry-matter (%)
0	1.91±0.03a	8.99±0.05a	34.0±0.8b	10.7±0.1a	18.0±0.2a
3	1.87±0.03a	7.97±0.06b	42.4±1.0a	10.6±0.1a	16.0±0.1b
6	1.94±0.06a	7.79±0.11b	ND	10.7±0.5a	15.1±0.3bc
9	1.70±0.08b	6.60±0.07c	ND	10.6±0.1a	14.9±0.1c
Mean	1.85±0.04	7.83±0.22	38.2±1.9	10.7±0.7	16.0±0.7
$LSD_{0.05}$	0.14*	0.24**	3.4*	NS	1.0**

ND, not detected.

*,**Significant difference at P<0.05 or <0.01.

NS, no significant difference at P=0.05.

Data within a single column with the same letter indicates no significant (P<0.05) difference in quality parameter with reference to storage duration.

Increases in off-flavours in MAP diced onion headspace[10,15] presumably contributed to the observed trends for separation of E-nose data set clusters in the PCA map and dendrogram (Figs 1 and 2). Volatiles production by spoilage organisms,[17] loss through the semi-polyethylene film of low molecular weight aromatic sulphur volatiles that comprise onion flavour, degradation of flavour compounds by enzymes and/or reduced alliinase activity due to prolonged storage at low temperature,[29] and metabolic switches from aerobic to anaerobic respiration[16] can all increase production of off-flavours. Such processes apparently differentially influenced responses of the E-nose sensor elements over time.

Nonetheless, relative changes in the E-nose sensor resistance ratio (%dR/R) of the 32-conducting polymer sensor were not significantly (P>0.05) different for days 0, 3 and 6 (Table 2). However, a small but significant (P<0.05) difference in %dR/R

was recorded for day 9. Thus, the PCA-based approach to analysing the data was the more sensitive method.

Total pyruvic acid content in sealed diced onion reduced with increasing storage duration (Table 2). Percentage reductions in pyruvic acid contents on days 3, 6 and 9 as compared to day 0 were 11.6, 13.3 and 26.6, respectively. The difference between days 3 and 6 was not significant ($P>0.05$). There was a positive linear association between total pyruvic acid (flavour) content (X) versus %dR/R (Y); where $Y = 1.166 + 0.879X$; $r = 0.803$; $P>0.05$; $n = 4$.

Lachrymatory potency as determined by time-intensity sensory data for hotness reduced significantly ($P<0.05$) from 34 s response time on day 0 to 40 s on day 3 (Table 2). After day 3, lachrymatory potency was completely lost. The lachrymatory factor, thiopropanal sulphoxide, is known to be unstable. It either vaporised and escaped the tissue at low temperature or was chemically converted to trans-3,4-diethyl-1,2-dithietane 1,1-dioxide.[1]

The total soluble solids content of the diced onion did not change over 9 days of storage (Table 2). Blanchard *et al*[11] observed that an increase in CO_2 concentration reduced physiological activity in diced onion stored for 14 days in air and in a controlled atmosphere system at 10% CO_2 with or without 2% O_2. Percentage dry-matter content significantly ($P<0.05$) reduced with increasing storage period (Table 2). The greatest reduction in dry-matter content from 18 to 16% was recorded after 3 days of storage. At this early time, dry-matter might have been consumed in increased physiological and biochemical activities following the severe tissue wounding.

In summary, a correlation was shown to exist between the E-nose data and more conventional means of determining the flavour quality of diced onion. This correlation suggests the E-nose could be used in commerce for assessment of minimally processed onion quality. The E-nose could save time, reduce financial cost, provide reliable results and offer relative ease of use.

ACKNOWLEDGEMENT

The authors thank Parripak Foods Ltd., UK for providing the diced onion packages used in this study.

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CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

Production and consumer demand for intact (FAOSTAT, 2002) and minimally processed vegetables (Resurreccion and Prussia, 1986), including Alliums, increase annually. These increasing trends, in addition to limitations on traditional sensory and analytical determinations of *Allium* quality (Sections 2.5.1 and 2.5.2), prompted scientists and institutions to develop and/or adopt new techniques to improve *Allium* quality evaluation (Sections 2.5.2 and 2.5.3). Relatively novel electronic nose (E-nose) technology is reported to be rapid, low cost and easy to use; thereby enabling near real-time discrimination of various products compared to traditional quality control methods (Payne, 1998; Giese, 2000). E-noses have been used for discrimination of genotypes and quality among various agricultural products, but not Alliums. Failure to discriminate among onion cultivars using an E-nose in previous unpublished work (B. Smith, pers. comm., 2000) may be ascribed to rapid and continuous changes in biochemical constituents in the homogenate. Thus, development of processing, handling and evaluation techniques to enhance E-nose sensor response and to minimise deviations in data sets output (Section 2.4.4) could benefit the *Allium* industry and improve discriminatory evaluation among *Allium* germplasm and between treatment samples from agronomic work. In view of this proposition, investigations were carried out to determine the potential for use of a conducting polymer sensor E-nose for discrimination among Alliums as affected by genotype, preharvest soil factors and postharvest storage duration. Relationships were examined between E-nose data set outputs; namely, proportional (%) changes in sensor resistance ratio (%dR/R) and Principal Components (PCs) versus analytical determination of pyruvic acid concentration (onion flavour strength index), sensory analysis of lachrymatory potency, % dry-matter and total soluble solids content (Chapters 3 to 5).

6.1.1 E-nose potential for discrimination of *Allium* headspace volatiles

The results of all experiments (Chapters 3 to 7) showed that *Allium* quality characteristics can be discriminated both at harvest and postharvest using a

conducting polymer sensor type E-nose. Addition of 5% trichloroacetic acid (TCA) to *Allium* homogenate was carried out to terminate alliinase hydrolysis of the endogenous alken(yl)-L-cysteine sulphoxide (ACSO) flavour precursor compounds (Schwimmer and Weston, 1961; Randle and Bussard, 1993b). Accordingly, organo-sulphur compounds in *Allium* homogenates and, presumably, headspace volatile compounds other than the highly volatile tear stimulant thiopropanal sulphoxide were stabilised (Schwimmer and Weston, 1961; Randle and Bussard, 1993b). Thus, the practical potential of meaningfully using the E-nose sensor response to discriminate among *Allium* headspace volatiles from different backgrounds was enhanced by the addition of 5% TCA compared to no addition of 5%TCA treatment (*Appendix* V). Similarly, Schwimmer and Weston (1961) employed the use of an electronic oven at a radio frequency energy of wavelength (λ) 12 cm for 5 min to deactivate alliinase.

Principal Component Analysis (PCA) is a pattern recognition method that estimates correlation structure within variables (Wold *et al.*, 1987). Application of PCA was useful in that it explained over 75% to nearly 100% of total variance in E-nose data sets for *Allium* headspace volatiles. Headspace flavour and non-flavour volatiles vary among different *Allium* genotypes (Freeman and Whenham, 1975c; Block *et al.*, 1992; Thomas and Parkin, 1994). Inter-specific (Chapter 3) and intra-specific (Chapter 4) variations in Alliums were for the first time detected using PCA classification of E-nose data sets as previously reported for coffee (*Coffea* spp.) by Aishima (1999), spices by Madsen and Grypa (2000) and mushroom (*Agaricus* spp.) by Keshri *et al.* (2003). Headspace volatiles of the different *Allium* types and varieties of a single *Allium* spp. differentially affected E-nose sensor conductivity measured in terms of %dR/R.

Variation in the responsiveness of *Allium* types, namely spring onion (Chapter 4) and bulb onion (Chapter 5), to nitrogen (N) and sulphur (S) fertilisation, irrigation regime and soil type treatments were apparent using the conducting polymer sensor E-nose. Variation in *Allium* response to preharvest soil treatments were associated with variations in %dR/R and the relative positions and magnitude of separations amongst E-nose data sets clusters in PCA plots as attested by the Mahalanobis distance (D^2) statistic (Mahalanobis, 1941; Gnanadesikan, 1977; Mark and Tunnell, 1985).

N and S fertilisation influences the composition and concentration of the onion flavour precursor ACSO, which in turn affects headspace flavour volatiles composition (Freeman and Mossadeghi, 1970; Randle *et al.*, 1994; 1995; Randle, 2000). Thus, hydrolysis of ACSO and, possibly, other non-flavour volatiles in the headspace for example, sulphides and some thiols (Block, 1992) can explain changes in %dR/R and classification of E-nose data sets in the PCA maps. Similarity in headspace volatiles between certain treatments is shown by overlap of data set clusters in the PCA maps (Sinesio *et al.*, 2000; Keshri *et al.*, 2003). Flavour-derived compounds vary for bulb onion versus spring onion (Section 2.3.5.1) and, thus, can be differentially influenced by fertiliser treatments. E-nose evaluation of green leaf bases plus immature bulb portions of glasshouse-grown spring onion cv. White Lisbon was not significantly ($P>0.05$) affected by S fertilisation (Section 4.2). In contrast, headspace volatiles of S-fertilised mature bulbs of onion cv. Sprinters grown in field and glasshouse experiments gave significantly ($P<0.05$) reduced %dR/R values upon interaction with E-nose sensor polymer (Section 5.1). Plant S partitioning is dependent on stage of maturity (Lancaster *et al.*, 1986). This suggests that S accumulation as cysteine sulphoxide in the mature bulb of onion cv. Sprinters led to the significant ($P<0.05$) reduction in E-nose sensor conductivity.

Increases in water-deficit stress reduce uptake of nutrients and metabolic processes (Lambers *et al.*, 1998; Glenn, 2000). The adverse effects of this water-deficit stress reduces assimilate production including cysteine sulphoxide on both clay and sandy loam soils. These explain the reductions in E-nose sensor conductivity and data set cluster separations between clay versus sandy loam by factors of 0.11 and 0.85, respectively, as water-deficit stress increased from -0.01 to -1.19 MPa soil water potential (Section 4.3). Consistent and significant ($P<0.05$) reductions in %dR/R values for the clay as compared with the sandy loam in all three experiments confirmed that variations in soil properties differentially affected headspace volatiles of both spring onion cv. White Lisbon and onion bulb cv. Sprinters.

The E-nose discriminated gradual but significant ($P<0.05$) changes in the quality characteristics of cut onions wrapped in polyethylene bags as storage duration was extended from 0 to 9 days at 0°C (Chapter 6). This effect was demonstrated by a 0.11-fold reduction in %dR/R after 9 days for headspace volatile molecules

interacting with the E-nose sensor polymer material. Similar findings were made on stored intact tomato (Sinesio *et al.*, 2000) and grains (Magan and Evans, 2000) using E-noses. Volatiles emissions by spoilage microorganisms in stored produce are also discriminated by E-nose assessment during storage (Keshri *et al.*, 1998), which could be extended to Alliums.

This study shows that it is possible to use a 32 conducting polymer sensor E-nose to discriminate among *Allium* germplasm lines. Also, *Allium* samples either preharvest or from storage can be discriminated by matching against a quality standard. Artificial Neural Network (ANN) is a pattern recognition system that compares samples to standards and, thus, mimics the memory storage system of the human brain (Payne, 1998). Thus, automatic E-nose discrimination of samples could be achieved using ANN.

6.1.2 Physiological quality variables

Allium pungency is usually measured as pyruvic acid concentration due to typically linear relationships between both ACSO and thiosulphinates versus pyruvic acid (Schwimmer and Weston, 1961; Freeman and McBreen, 1973; Thomas and Parkin, 1994). Pyruvic acid is a stable, non-flavour by-product formed during hydrolysis of ACSOs. In conformity with other published work (Freeman and Mossadeghi, 1970; Hamilton *et al.*, 1998; Randle *et al.*, 1994; 1999), an increase in S fertiliser amounts to increased pyruvic acid concentration and lachrymatory potency of Alliums (Chapters 4 and 5). However, in a single variety (cv. White Lisbon) trial, an increase in S fertiliser rate from 2.9 to 5.8 kg ha^{-1} diminished the relative amount of increase in pyruvic acid concentration as compared with pyruvic acid concentration following an increase in S rate from 0 to 2.9 kg ha^{-1} (Section 4.2). Pyruvic acid concentrations in field-grown onion bulb cv. Sprinters were *ca* 5% greater for the no fertiliser added treatment compared to addition of both N and S. This finding can be ascribed to greater intrinsic pyruvic acid concentration (not determined) in those plants that were not supplied with N nor S fertiliser. The greater concentration of pyruvic acid for the glasshouse clay treatment compared to the glasshouse sandy loam treatment is in contrast with report by Mohamed *et al.* (1993) and Hamilton *et al.* (1998). They reported that soil type did not affect pyruvic acid concentration of onions. Nonetheless, the high N and S concentrations of the clay compared to the sandy loam

soil (Section 4.1) before the experiment might have brought about the significant ($P<0.05$) difference in pyruvic acid concentration.

Effects of water-deficit stress on onion quality were indicated by significant ($P<0.05$) irrigation x soil type interactions for pyruvic acid concentration, TSS and % dry-matter contents of spring onion cv. White Lisbon (Section 4.3). These quality indices were largely reduced on the sandy loam due to low water storage capacity as compared with the clay soil (Glenn, 2000). N and S fertilisation and the clay soil reduced total soluble solids content of spring and bulb onions (Section 4.2 and Chapter 5).

All biomolecules including growth, defence and storage compounds are made up of carbon (C) skeletons in addition to hydrogen (H) and oxygen (O) (Stryer, 1998). An onion bulb is a storage organ that comprises compounds such as sugars, proteins and cysteine sulphoxides. Due to the importance of cysteine sulphoxides as storage compounds and their involvement in plant defence mechanisms, there seems to be high affinity for the use of N and S in cysteine sulphoxide pathways compared to the use of N and S for protein synthesis (i.e. growth). Such preference can reduce the amount of C skeletons available for production of other compounds such as carbohydrates. From the results of the present study, it appears there may have been a trade-off between pyruvic acid concentration and TSS content. Following N and S fertilisation pyruvic acid concentration increased, probably due to increased concentration of cysteine sulphoxide. There was a corresponding reduction in TSS content, which could be ascribed to reductions in soluble compounds such as carbohydrates typically fructans, soluble sugars and some amino acids. These findings agree with work by Randle (1992a) and Randle and Bussard (1993a).

For cut onions wrapped in polyethylene bags, pyruvic acid concentration and %DM content were reduced by 0.11- and 0.25-fold, respectively, after 3 days of storage at 4°C compared to day 0 (Chapter 6). Reduction in alliinase activity and/or concentration due to prolong low temperature storage (Lewis *et al.*, 1977; Benkeblia, 2000), escape of low molecular weight organosulphur compounds and degradation of cysteine sulphoxides for defense against disease pathogens (Kyung *et al.*, 2002) can all reduce pyruvic acid concentration in the diced onions during storage. Thus, dry-

matter may have been reduced through being utilised during maintenance respiration as part of the wound healing process (Howard *et al.*, 1994; Blanchard *et al.*, 1996), hence its reduction after day 3. However, TSS content did not change during the 9 days storage period. This result agrees with previous report for total sugars in diced onion bulbs, which did not change after 14 days at 4°C (Blanchard *et al.*, 1996).

6.1.3 Plant growth

Increases in leaf greenness (chlorophyll concentration) with additional N and S can increase assimilate synthesis (Lambers *et al.*, 1998). Consequently, plant growth and yield parameters of leaf elongation, fresh and dry weights of plant (foliage and bulb) and yield of spring onions (Chapter 4) and bulb onion cv. Sprinters (*Appendix* III) were increased. Enhancement of spring onion water-use efficiency, relative water content, leaf tissue water potential and DM accumulation depends on increased soil water availability (Lambers *et al.*, 1998; Glenn, 2000). Thus, the incremental effects consequently improved plant growth and spring onion yield on regularly watered plots at -0.01 MPa soil water potential, especially on the clay compared to the sandy loam (Section 4.3).

In comparison with the sandy loam, the inherent properties of the clay, including high cation exchange capacity, better textural and structural characteristics and optimum soil water availability (Brady and Weil, 1996; Rowell, 1996), significantly ($P<0.05$) increased growth, yield, and bulb skin quality. This finding agrees with other reports on bulb onion by Talha (1978) and Mohamed *et al.* (1993) and on garlic by Hanson *et al.* (2003). As previously reported by Brouwer (1963), Creelman *et al.* (1990) and Hsiao (2000), shoot-to-root fresh weight ratio was not influenced by any of the preharvest soil variables nor their interactions.

6.1.4 Microbial load

Microbial load was significantly ($P<0.01$) lower in the inner tissues of S-fertilised bulbs (Section 5.1). Microbial loads were 0.80-, 0.92- and 0.27-fold lower in S-fertilised bulbs harvested from the field, glasshouse clay and glasshouse sandy loam, respectively, compared with S-deficient bulbs. S fertilisation increased onion flavour, which suggested an increase in ACSO and, therefore, potential anti-microbial properties (Block, 1992; Kyung *et al.*, 2002). Degradation of cysteine and

glutathione, both of which are primary compounds for ACSO synthesis, produce elemental S that can protect plants against disease pathogens as reported for tomato (Williams *et al.*, 2002). Bulbs harvested from the clay also had higher flavour content and 75% less microbial load compared with bulbs harvested from the sandy loam.

6.2 Overall Conclusions

This work has established and confirmed the potential for use of an E-nose for discrimination of inter- and intra-specific variations in *Allium* spp. Genetic variability, variations in soil type and the availability of soil mineral nutrients of N and S, and soil moisture content each influenced the headspace volatiles of onions (Fig. 1). Thus, differential modulation of the E-nose polymer sensor electric conduction units gave electronic fingerprints that semi-quantitatively characterised sample headspace volatiles. The findings demonstrate that the use of an E-nose could facilitate *Allium* germplasm evaluation in breeding work, discrimination of agro-physiological attributes and monitor quality of *Allium* along postharvest and marketing chains.

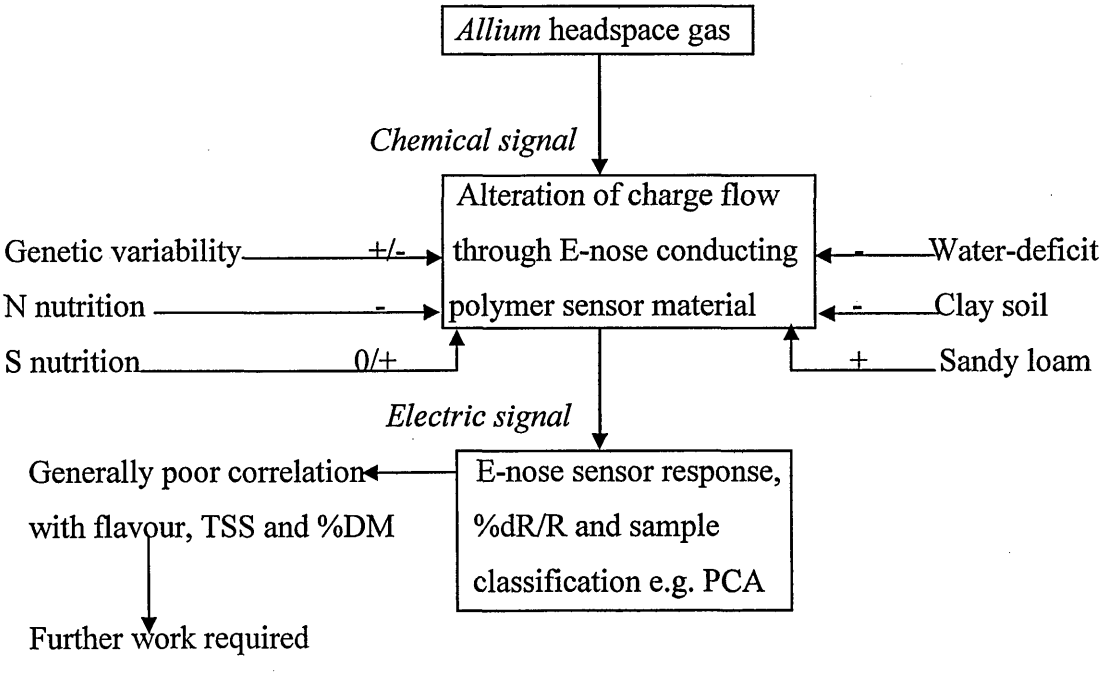

Figure 1. Outline of generalised response of an E-nose conducting polymer sensor element to *Allium* headspace volatiles affected by genotype and edaphic factors. + for increased effect; - for reduced effect; +/- for variable effect and 0 for no effect on %dR/R.

Electric charge flow through the E-nose sensor polymer was differentially influenced by *Allium* genotypes. N nutrition, water-deficit stress and the clay treatment reduced %dR/R, which suggest an increase in charge flow as compared with the sandy loam treatment, which increased %dR/R. For spring onions, S nutrition had no effect on %dR/R but %dR/R value was reduced for S-fertilised bulb onion cv. Sprinters.

The E-nose can operate at a comparatively shorter period of time per unit sample than it takes either to operate instruments such as the GC-MS or to conduct sensory (taste) panel evaluation. E-nose assessment of *Allium* quality requires minimal resource input compared with conventional analytical and sensory tests. Thus, the E-nose can also augment analytical determination and sensory appraisal of *Allium* flavour. Analytical methods are normally constrained by high cost, longer time for sample analysis and specialist training (Payne, 1998; Giese, 2000). Longer time for organisation and training of panellists, inherent limitations of panellists (Manley, 1993; Payne, 1998), and inconsistency in results of onion sensory evaluations (D. O'Connor, Allium and Brassica Centre, pers. comm., 2002) limits the use of sensory taste panel even though it is the most important and commonly used test.

Although the 32 conducting polymer sensor E-nose discerned treatment differences in the present work, the E-nose sensor responses and pattern of data sets cluster locations in PCA plots did not correlate with traditional measures of sensory and instrumental analyses of *Allium* flavour (Fig. 1). The poor correlation suggests that unlike sensory evaluation, the E-nose sensor elements did not interact with flavour-active molecules alone in the *Allium* headspace. They presumably interacted with both flavour and non-flavour molecules in the headspace. Further investigation is needed to identify the actual chemicals in *Allium* headspace that interacted with the E-nose sensor element. Thus, ongoing projects to develop chemical-specific E-nose sensors for various industries (Barker *et al.*, 2002) should include Alliums.

Onion growth and flavour (including pungency) were reduced by imbalanced N/S nutrition and large fluctuations in soil moisture levels. For instance, onion tissue N:water-soluble SO_4^{2-} ratios between 5 and 8 were associated with greater %dR/R values and flavour. For greater growth and yield and better bulb scale leaf quality, tissue N:water-soluble SO_4^{2-} ratios of 5 were found in the field and glasshouse sandy

loam soils and of 18 was found for the glasshouse clay. The differences in onion tissue N:water-soluble SO_4^{2-} ratios required for higher growth and yield can be ascribed to variations in properties between the clay and the sandy loam soils (Brady and Weil, 1996; Rowell, 1996).

This work confirmed increases in flavours of eight spring onion cultivars and bulb onion cv. Sprinters upon additional S fertilisation as reported by previous workers (Freeman and Mossadeghi, 1970; Randle *et al.*, 1993; 1995). At low S, the demand for mild onions in Europe and the USA (Smittle *et al.*, 1979) most probably can be met by selection of cultivars that thrive under low S levels. A typical example of such a mild onion is the recently developed "Supasweet" onion (Cook and Smith, 2002). Where high rate of S is applied to increase onion growth and yield and to reduce disease incidence, it may be possible to use an E-nose in combination with an ANN as a pattern recognition system to select mild onions by screening a wide range of cultivars.

Overall, growth and quality of onions harvested from the clay soil were greater than those harvested from the sandy loam. Clay soils in general, have greater proportions of pH-dependent cation exchange capacity, which improves soil nutrient availability and soil water retention capacity (Rowell, 1996; Brady and Weil, 1996). Sandy soils can be managed by addition of organic matter to improve soil humus and water-holding capacity. Amendment of soil pH using chemical fertilisers may also enhance soil nutrient availability, which normally increase onion growth and yield (Sullivan *et al.*, 2001).

6.3 Areas for Future Research

Based on this work, the following suggestions are made for future studies on E-nose assessment of onion headspace flavour and non-flavour volatiles. Also, suggestions for further experiments in agronomy and postharvest are presented.

E-nose evaluation versus analytical tests

i) Generally, there was no association between PCA classifications of E-nose data set or %dR/R versus analytical or sensory determination of flavour.

Thus, further work is required to quantitatively determine chemical composition in the *Allium* headspace volatiles that interact with the sensor polymer using GC-MS.

ii) Optimisation of E-nose sensors specific for *Allium* quality assessment would be beneficial to workers undertaking onion germplasm evaluation and to the onion industry.

iii) Work is needed to reduce sample preparation time and eliminate destructive sampling as used for the E-nose evaluation in this study.

Preharvest and postharvest studies

i) Physiological explanation is required for the low %dR/R and pyruvic acid content of spring onion cv. White Lisbon following regular watering at -0.01 MPa soil water potential on sandy loam compared with regular watering on the clay and water-deficit stressed plants on both clay and sandy loam.

ii) Investigate the potential for use of E-nose to monitor spoilage and disease pathogens of Alliums during storage.

iii) A study to investigate N, S and soil type effects, and their interactions on onion storage ability will be beneficial. Such work should investigate trade-offs between pungency, sugar concentrations and growth.

CHAPTER 7: REFERENCES

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APPENDICES

Appendix I: ANOVA Tables

CHAPTER 3

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Allium spp.	4	0.22888	0.05722	44.79	0.000
Error	20	0.02555	0.00128		
Total	24	0.25443			

$[\%dR/R + 0.5]^{0.5}$ (repeat)

Source	DF	SS	MS	F	P
Allium spp.	4	0.18876	0.04719	15.76	0.000
Error	20	0.05990	0.00300		
Total	24	0.24866			

Total soluble solids

Source	DF	SS	MS	F	P
Allium spp.	4	464.923	116.231	761.34	0.000
Error	10	1.527	0.153		
Total	14	466.449			

Total pyruvic acid

Source	DF	SS	MS	F	P
Allium spp.	4	6761.89	1690.47	299.91	0.000
Error	10	56.37	5.64		
Total	14	6818.26			

Background pyruvic acid

Source	DF	SS	MS	F	P
Allium spp.	4	2981.47	745.37	102.57	0.000
Error	10	72.67	7.27		
Total	14	3054.14			

$[\%DM]^{0.5}$

Source	DF	SS	MS	F	P
Allium spp.	4	2170.176	542.544	825.18	0.000
Error	15	9.862	0.657		
Total	19	2180.038			

Regression Analysis

Pyruvic acid = 14.4 + 8.53 PC 1 + 18.2 PC 2

Source	DF	SS	MS	F	P
Regression	2	289.63	144.82	8.62	0.104
Residual Error	2	33.60	16.80		
Total	4	323.23			

Total soluble solids = 7.09 + 6.33 PC 1 + 12.0 PC 2

Source	DF	SS	MS	F	P
Regression	2	148.418	74.209	25.28	0.038
Residual Error	2	5.870	2.935		
Total	4	154.288			

%Dry-matter = 16.0 + 12.1 PC 1 + 22.5 PC 2

Source	DF	SS	MS	F	P
Regression	2	533.82	266.91	28.61	0.034
Residual Error	2	18.66	9.33		
Total	4	552.48			

CHAPTER 4 (SECTION 4.1)

Bulb diameter

Source	DF	SS	MS	F	P
Block	4	24.381	6.095	2.48	0.054
Cultivar	7	681.713	97.388	39.57	0.000
Cultivar*S	7	112.174	16.025	6.51	0.000
S	1	7.688	7.688	3.12	0.082
Error	60	147.677	2.461		
Total	79	973.633			

Log_{10} [no. of green leaf]

Source	DF	SS	MS	F	P
Block	4	0.020554	0.005138	1.32	0.273
Cultivar	7	0.203547	0.029078	7.46	0.000
Cultivar*S	7	0.016099	0.002300	0.59	0.761
S	1	0.137589	0.137589	35.31	0.000
Error	60	0.233799	0.003897		
Total	79	0.611587			

Leaf length

Source	DF	SS	MS	F	P
Block	4	13.814	3.453	0.54	0.705
Cultivar	7	97.163	13.880	2.18	0.049
Cultivar*S	7	33.370	4.767	0.75	0.632
S	1	322.003	322.003	50.60	0.000
Error	60	381.795	6.363		
Total	79	848.145			

SPAD value of leaf greenness

Source	DF	SS	MS	F	P
Block	4	147.17	36.79	0.48	0.750
Cultivar	7	1446.99	206.71	2.70	0.017
Cultivar*S	7	583.19	83.31	1.09	0.383
S	1	6248.11	6248.11	81.50	0.000
Error	60	4600.03	76.67		
Total	79	13025.49			

SPAD value of leaf greenness

Source	DF	SS	MS	F	P
Block	4	153.1	38.3	0.95	0.454
soil	1	93.6	93.6	2.33	0.142
soil*S	2	6.1	3.0	0.08	0.928
S	2	832.2	4916.1	122.40	0.000
Error	20	803.3	40.2		
Total	29	10888.3			

Plant height

Source	DF	SS	MS	F	P
Block	4	60.40	15.10	0.70	0.602
soil	1	0.77	0.77	0.04	0.852
soil*S	2	29.02	14.51	0.67	0.523
S	2	296.85	1398.43	64.62	0.000
Error	20	432.78	21.64		
Total	29	3319.82			

Shoot:root fresh weight ratio

Source	DF	SS	MS	F	P
Block	2	0.0793	0.0397	0.21	0.815
soil	1	0.1168	0.1168	0.61	0.451
soil*S	2	0.0487	0.0243	0.13	0.881
S	2	0.2743	0.1371	0.72	0.510
Error	10	1.9002	0.1900		
Total	17	2.4192			

SECTION 4.2

Pyruvic acid

Source	DF	SS	MS	F	P
Block	3	0.9015	0.3005	2.89	0.046
Cultivar	7	20.0504	2.8643	27.52	0.000
Cultivar*S	7	12.9974	1.8568	17.84	0.000
S	1	127.3512	127.3512	1223.77	0.000
Error	45	4.6829	0.1041		
Total	63	165.9834			

Pyruvic acid

Source	DF	SS	MS	F	P
Block	3	0.792	0.264	3.09	0.059
soil	1	0.000	0.000	0.00	0.967
soil*S	2	6.854	3.427	40.08	0.000
S	2	253.178	126.589	1480.46	0.000
Error	15	1.283	0.086		
Total	23	262.107			

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Block	4	0.007125	0.001781	0.90	0.482
soil	1	0.010457	0.010457	5.29	0.032
soil*S	2	0.019066	0.009533	4.82	0.020
S	2	0.005631	0.002816	1.42	0.264
Error	20	0.039552	0.001978		
Total	29	0.081832			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	2.3292	0.7764	4.40	0.008
Cultivar	7	42.3461	6.0494	34.31	0.000
Cultivar*S	7	21.0023	3.0003	17.02	0.000
S	1	15.5039	15.5039	87.94	0.000
Error	45	7.9333	0.1763		
Total	63	89.1148			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	2.7283	0.9094	12.16	0.000
soil	1	4.5067	4.5067	60.27	0.000
soil*S	2	0.0858	0.0429	0.57	0.575
S	2	7.9825	3.9912	53.37	0.000
Error	15	1.1217	0.0748		
Total	23	16.4250			

$[\%DM]^{0.5}$

Source	DF	SS	MS	F	P
Block	2	0.11814	0.05907	1.10	0.371
soil	1	0.00014	0.00014	0.00	0.960
soil*S	2	0.02698	0.01349	0.25	0.783

S	2	0.18898	0.09449	1.76	0.222
Error	10	0.53806	0.05381		
Total	17	0.87229			

CHAPTER 4.3

Shoot:root fresh weight ratio

Source	DF	SS	MS	F	P
Block	3	0.5019	0.1673	1.05	0.398
Soil	1	0.2243	0.2243	1.41	0.253
Irrigation	2	0.7727	0.3863	2.43	0.122
Soil*irrigation	2	0.0773	0.0387	0.24	0.787
Error	15	2.3847	0.1590		
Total	23	3.9608			

Leaf water potential for the clay treatment

Source	DF	SS	MS	F	P
Block	4	0.004047	0.001012	0.13	0.970
Soil	1	0.032670	0.032670	4.15	0.055
Soils*irrigation	2	0.011540	0.005770	0.73	0.493
Irrigation	2	0.228407	0.114203	14.51	0.000
Error	20	0.157433	0.007872		
Total	29	0.434097			

Leaf water potential for the sandy loam treatment

Source	DF	SS	MS	F	P
Block	3	0.014483	0.004828	1.95	0.165
Soil	1	0.180267	0.180267	72.85	0.000
Irrigation	2	0.259358	0.129679	52.41	0.000
Soil*irrigation	2	0.247758	0.123879	50.06	0.000
Error	15	0.037117	0.002474		
Total	23	0.738983			

Relative tissue water content for the clay treatment

Source	DF	SS	MS	F	P
Block	4	0.0077200	0.0019300	1.95	0.141
Soils	1	0.0038533	0.0038533	3.90	0.062
Soils*irrigation	2	0.0073267	0.0036633	3.71	0.043
Irrigation	2	0.0432600	0.0216300	21.89	0.000
Error	20	0.0197600	0.0009880		
Total	29	0.0819200			

Relative tissue water content for the sandy loam treatment

Source	DF	SS	MS	F	P
Block	4	0.0027667	0.0006917	0.88	0.492
Soil	1	0.0007500	0.0007500	0.96	0.340
Soil*irrigation	2	0.0022400	0.0011200	1.43	0.263
Irrigation	2	0.0009867	0.0004933	0.63	0.543
Error	20	0.0156733	0.0007837		
Total	29	0.0224167			

Log [no. of green leaf]

Source	DF	SS	MS	F	P
Block	2	0.001280	0.000640	0.63	0.554
Soil	1	0.026877	0.026877	26.32	0.000
Irrigation	2	0.014816	0.007408	7.25	0.011
soil*irrigation	2	0.005164	0.002582	2.53	0.129
Error	10	0.010213	0.001021		
Total	17	0.058349			

Leaf length

Source	DF	SS	MS	F	P
Block	2	6.840	3.420	2.15	0.167
Soil	1	143.200	143.200	89.94	0.000
Irrigation	2	188.669	94.334	59.25	0.000
Soil*irrigation	2	3.905	1.952	1.23	0.334
Error	10	15.922	1.592		
Total	17	358.535			

Water-use efficiency

Source	DF	SS	MS	F	P
Block	2	0.017911	0.008956	4.60	0.038
Soil	1	0.002450	0.002450	1.26	0.288
Soil*irrigation	2	0.070233	0.035117	18.02	0.000
Irrigation	2	0.039211	0.019606	10.06	0.004
Error	10	0.019489	0.001949		
Total	17	0.149294			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	0.1633	0.0544	0.86	0.482
Soil	1	2.0417	2.0417	32.35	0.000
Irrigation	2	42.7558	21.3779	338.73	0.000
Soil*irrigation	2	1.5058	0.7529	11.93	0.001
Error	15	0.9467	0.0631		
Total	23	47.4133			

Time-intensity of lachrymatory potency

Source	DF	SS	MS	F	P
Block	3	288.33	96.11	1.92	0.169
Soil	1	793.50	793.50	15.87	0.001
Irrigation	2	91.00	45.50	0.91	0.424
Soil*Irrigation	2	237.00	118.50	2.37	0.128
Error	15	750.17	50.01		
Total	23	2160.00			

Pyruvic acid

Source	DF	SS	MS	F	P
Block	3	0.0158	0.0053	0.03	0.992
Soil	1	5.6843	5.6843	34.25	0.000
Irrigation	2	3.7230	1.8615	11.22	0.001
Soil*irrigation	2	10.3697	5.1848	31.24	0.000
Error	15	2.4892	0.1659		
Total	23	22.2819			

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Block	4	0.0057432	0.0014358	1.92	0.147
Soils	1	0.0001439	0.0001439	0.19	0.666
Soils*irrigation	2	0.0018813	0.0009407	1.26	0.306
Irrigation	2	0.0216423	0.0108212	14.44	0.000
Error	20	0.0149829	0.0007491		
Total	29	0.0443936			

CHAPTER 5 (SECTION 5.1)
FIELD EXPERIMENT

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Block	3	0.00455	0.00152	0.24	0.868
N	1	0.07048	0.07048	11.04	0.009

S	1	0.45074	0.45074	70.60	0.000
N*S	1	0.33198	0.33198	52.00	0.000
Error	9	0.05746	0.00638		
Total	15	0.91520			

Time-intensity of lachrymatory potency

Source	DF	SS	MS	F	P
Block	3	21.250	7.083	2.48	0.128
N	1	182.250	182.250	63.70	0.000
S	1	306.250	306.250	107.04	0.000
N*S	1	56.250	56.250	19.66	0.002
Error	9	25.750	2.861		
Total	15	591.750			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	1.5269	0.5090	1.82	0.213
N	1	9.1506	9.1506	32.74	0.000
S	1	4.3056	4.3056	15.40	0.003
N*S	1	6.8906	6.8906	24.65	0.001
Error	9	2.5156	0.2795		
Total	15	24.3894			

Microbial load

Source	DF	SS	MS	F	P
Block	2	0.04525	0.02263	0.27	0.769
N	1	0.35694	0.35694	4.32	0.083
S	1	2.26879	2.26879	27.46	0.002
N*S	1	0.48876	0.48876	5.92	0.051
Error	6	0.49576	0.08263		
Total	11	3.65549			

Regression Analysis

Pyruvic acid = 12.2 - 0.0553 PC 1 + 0.07 PC 2

Source	DF	SS	MS	F	P
Regression	2	1.4665	0.7332	1.48	0.263
Residual Error	13	6.4258	0.4943		
Total	15	7.8923			

Lachrymatory potency = 18.2 + 0.503 PC 1 - 1.10 PC 2

Source	DF	SS	MS	F	P
Regression	2	123.33	61.67	1.71	0.219
Residual Error	13	468.42	36.03		
Total	15	591.75			

Total soluble solids = 10.7 + 0.0306 PC 1 + 0.76 PC 2

Source	DF	SS	MS	F	P
Regression	2	0.560	0.280	0.15	0.860
Residual Error	13	23.829	1.833		
Total	15	24.389			

GLASSHOUSE EXPERIMENT

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Block	3	0.04888	0.01629	2.37	0.099
Soil	1	0.18186	0.18186	26.48	0.000
N	1	0.01699	0.01699	2.47	0.131
S	1	0.31478	0.31478	45.84	0.000
Soil*N	1	0.98399	0.98399	143.28	0.000

Soil*S	1	0.02334	0.02334	3.40	0.079
N*S	1	0.00293	0.00293	0.43	0.521
Soil*N*S	1	0.04260	0.04260	6.20	0.021
Error	21	0.14422	0.00687		
Total	31	1.75959			

Pyruvic acid

Source	DF	SS	MS	F	P
Block	3	0.025	0.008	0.81	0.504
Soil	1	6.817	6.817	666.99	0.000
N	1	13.559	13.559	1326.59	0.000
S	1	79.160	79.160	7744.82	0.000
Soil*N	1	8.010	8.010	783.68	0.000
Soil*S	1	2.055	2.055	201.09	0.000
N*S	1	0.004	0.004	0.42	0.525
Soil*N*S	1	3.348	3.348	327.52	0.000
Error	21	0.215	0.010		
Total	31	113.193			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	0.4237	0.1412	0.35	0.791
Soils	1	25.9200	25.9200	63.92	0.000
N	1	0.0013	0.0013	0.00	0.956
S	1	1.6200	1.6200	3.99	0.059
Soils*N	1	3.3800	3.3800	8.33	0.009
Soil*S	1	0.9113	0.9113	2.25	0.149
N*S	1	0.2450	0.2450	0.60	0.446
Soil*N*S	1	2.1013	2.1013	5.18	0.033
Error	21	8.5163	0.4055		
Total	31	43.1188			

Microbial load

Source	DF	SS	MS	F	P
Block	2	0.0023	0.0011	0.05	0.953
Soil	1	2.6667	2.6667	113.73	0.000
N	1	0.0687	0.0687	2.93	0.109
S	1	4.8216	4.8216	205.64	0.000
Soil*N	1	0.2380	0.2380	10.15	0.007
Soil*S	1	3.5411	3.5411	151.03	0.000
N*S	1	0.4427	0.4427	18.88	0.001
Soil*N*S	1	0.6935	0.6935	29.58	0.000
Error	14	0.3283	0.0234		
Total	23	12.8027			

Regression Analysis

Clay treatment

Pyruvic acid = 7.48 + 0.009 PC 1 + 5.69 PC 2

Source	DF	SS	MS	F	P
Regression	2	7.569	3.784	0.71	0.508
Residual Error	13	68.877	5.298		
Total	15	76.446			

Total soluble solids = 10.9 + 0.0732 PC 1 + 3.54 PC 2

Source	DF	SS	MS	F	P
Regression	2	5.2007	2.6004	5.50	0.019
Residual Error	13	6.1487	0.4730		
Total	15	11.3494			

Sandy loam treatment

Pyruvic acid = 6.58 - 0.0902 PC 1 - 1.39 PC 2

Source	DF	SS	MS	F	P
Regression	2	4.843	2.422	1.25	0.317
Residual Error	13	25.086	1.930		
Total	15	29.929			

Total soluble solids = 9.09 + 0.0360 PC 1 - 0.799 PC 2

Source	DF	SS	MS	F	P
Regression	2	0.9570	0.4785	1.27	0.313
Residual Error	13	4.8924	0.3763		
Total	15	5.8494			

SECTION 5.2

$[\%dR/R + 0.5]^{0.5}$

Source	DF	SS	MS	F	P
Block	7	0.22035	0.03148	1.73	0.155
Day	3	0.26214	0.08738	4.81	0.011
Error	21	0.38154	0.01817		
Total	31	0.86404			

Pyruvic acid

Source	DF	SS	MS	F	P
Block	7	0.2494	0.0356	0.70	0.674
Day	3	22.9136	7.6379	149.58	0.000
Error	21	1.0723	0.0511		
Total	31	24.2353			

Time-intensity of lachrymatory potency

Source	DF	SS	MS	F	P
Block	3	5.500	1.833	0.41	0.760
Day	1	84.500	84.500	18.78	0.023
Error	3	13.500	4.500		
Total	7	103.500			

$[\%DM]^{0.5}$

Source	DF	SS	MS	F	P
Block	3	0.1820	0.0607	0.16	0.920
Day	3	24.3480	8.1160	21.49	0.000
Error	9	3.3989	0.3777		
Total	15	27.9289			

Total soluble solids

Source	DF	SS	MS	F	P
Block	3	0.231875	0.077292	12.51	0.001
Day	3	0.031875	0.010625	1.72	0.232
Error	9	0.055625	0.006181		
Total	15	0.319375			

Appendix II

Electronic Nose-based Discrimination among Spring Onions Grown on Two Different Soils at Three Water-deficit Stress Levels

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Keywords: *Allium cepa*, clay, flavour, headspace volatiles, sandy loam, soil water potential

Abstract

Quality discrimination for spring onions using conventional methods of sensory appraisal and analytical tests is difficult, expensive and time-consuming. Discrimination of spring onion characteristics with electronic nose (E-nose) technology was investigated. Plants of cv. White Lisbon were grown in a glasshouse in pots containing clay (Alluvial gley) or sandy loam (Brown earth). Irrigation regimes were regular watering to near field capacity (-0.01 MPa soil water potential, SWP) or re-watering to near field capacity when available moisture level was depleted to either ≤50% (-0.80 MPa SWP) or ≤25% (-1.19 MPa SWP). The E-nose sensor response (%dR/R) was significantly ($P<0.01$) influenced by irrigation, with %dR/R decreasing in association with reducing soil water availability. Effects of soil type and irrigation regime x soil type interaction for %dR/R were not significant ($P>0.05$). Two-dimension Principal Component Analysis (PCA) plots showed significant ($D^2>3.0$) differences among data set clusters. Increases in water-deficit level reduced separations between data set clusters for plants grown on both clay and on sandy loam. Regular irrigation increased pyruvic acid concentration by 43% on the clay as compared with 8% increase in mild water-deficit stressed plants on clay versus severely stressed plants. In contrast, pyruvic acid concentration was reduced by 8% in regularly watered plants grown on the sandy loam as compared with 8% increase in mild water-deficit stressed plants on sandy loam versus severely stressed plants. In conclusion, significant ($D^2>3.0$) separations of data set clusters in association with water-deficit stress but not soil type were evident on the 2D PCA plots. However, while the E-nose has demonstrated potential for discrimination of spring onion quality, further detailed work is required to characterise the interactions of spring onion volatile components with conducting polymer sensors.

INTRODUCTION

Spring onion (*Allium cepa*, L.) is a tasty salad vegetable that is consumed worldwide due to volatile flavours released during chewing (Block, 1992). Onion flavour intensity is dependent on genetic variability modified by environmental factors (Randle, 1992; Hamilton et al., 1998). Excessive uptake of water by onion bulbs causes hydrolysis of fructans to free fructose, which in turn reduces soluble sugars and dry matter content (Darbyshire and Henry, 1979). On the other hand, mild water-deficit stress increases photosynthates and total soluble solids content (Kratky et al., 1990; Beverly et al., 1993). Biological, physical and chemical properties of the soil and management practices affect the quality of fresh produce, including flavour characteristics (Joyce, 1999). Differences in onion bulb flavour were found when plants were dependent upon inherent soil nutrient content (Mohammed et al., 1993; Hamilton et al., 1998). However, onion bulb pungency did not differ when the same amounts of nutrients were applied to plants grown on clay versus sandy loam (Mohammed et al., 1993).

Either biochemical determination of pyruvic acid or thiosulphinate concentration and/or organoleptic appraisal of lachrymatory (tear-inducing) potency (Freeman and Whenham, 1975) are conventionally used to measure onion flavour. However, these methods are difficult, expensive and time-consuming, especially when evaluating large

Proc. 8th Int. CA Conference
Eds. J. Oosterhaven & H.W. Peppelenbos
Acta Hort 600, ISHS 2003

numbers of samples.

The electronic nose (E-nose) is a relatively novel device used for qualitative volatiles sensing. This device incorporates inorganic crystalline or polycrystalline, organic or polymer, and biologically derived (Gardner and Bartlett, 1999) sensors that interact with molecules of headspace gas. For polymer sensors derived from pyrrole, aniline and thiophene monomers (Gardner and Bartlett, 1999), these interactions cause changes in sensor resistance to electron flow. As a result, a fingerprint of the odour is produced (Gopel et al., 1998). PCA maps are commonly used in association with E-nose technology for classification of headspace volatiles from agricultural, health, pharmaceutical and industrial products (Persaud and Talou, 1996; Bartlett et al., 1997; Sinesio et al., 2000). For muskmelon and tomato, Benady et al. (1995) and Sinesio et al. (2000) were able to interpret PCA maps of headspace volatiles data in terms of flavour. Smaller changes in a semiconductor gas sensor resistance ratio, Rs/Ro, where Rs = measured resistance and Ro = sensor resistance, under specified conditions of an electronic sniffer have been associated with higher volatiles intensity of muskmelon (Benady et al., 1995). The potential application of a conducting polymer sensor E-nose to evaluate quality of spring onions grown on two soil types with three different levels of irrigation was investigated in the present study.

MATERIALS AND METHODS

Five seedlings of spring onion cv. White Lisbon were transplanted into 12-cm diameter plastic pots in August 2000. Pots contained either clay (Alluvial gley series) or sandy loam (Brown earths series). Irrigation treatments were regular watering to near field capacity (-0.01 MPa soil water potential, SWP) and re-watering to near field capacity when soil moisture content was either ≤50% (-0.80 MPa SWP) or ≤25% (-1.19 MPa SWP) of available water holding capacity. The glasshouse experiment design was a randomised complete block factorial (soil type x irrigation level) with three blocks. Plants were harvested 16 weeks after transplanting. Edible portions of spring onions from each of the three blocks per treatment were bulked together and then subdivided into four (five for E-nose evaluation) replicate samples for quality assessment.

For E-nose evaluation, the edible portions were homogenised in a Moulinex (TIPO 753; Patendo, Spain) mixer at room temperature. A mixture of homogenate and 5% trichloroacetic acid (TCA) (1:1 v/v) was prepared. This mixture was diluted with deionised water (1:1 v/v). One ml of the diluted mixture was put into a 100 ml Schott bottle. The bottle was placed in the sample station of an AromaScan LabStation System A32/8S (Osmotech, UK) to equilibrate for 10 min at 25°C and 30% RH before sampling. Headspace gas was sampled for a period of 70 s at a gas flow rate of 50 ml min^{-1} at 25°C and 30% RH. Responses of the 32-conducting polymer sensor to headspace volatiles of the mixture for each treatment sample were analysed and processed using A32S Microsoft Windows Version 3.24B software (AromaScan Plc., UK). The E-nose sensor response was measured by %dR/R = [(Ro-Rs)/Ro] x 100, where Ro = original resistance and Rs = sensor resistance in the presence of the sample. %dR/R data were transformed by means of the square-root transformation rule before ANOVA (Gomez and Gomez, 1984) using Minitab for Windows version 12.23 software (Minitab Inc., USA). The original untransformed data are, however, presented for easy interpretation (Table 1). Two-dimension (2D) Principal Component Analysis (PCA) plots were also generated. Separations between centres of treatment data clusters on the 2D PCA plots were determined with A32S software by the Mahalanobis distance (D^2). The minimum D^2 classification rule assessed location of clusters (Morrison, 1976; Gnanadesikan, 1977). This states that an observation 'x' is assigned to a population 'i' if $Di^2<Dj^2$; 'i'≠'j'. A $D^2>3.0$ was considered as significant separation between clusters.

To determine pyruvic acid concentration, 10 ml of the homogenate/TCA mixture was filtered, made up with deionised water to the 200-ml level in a conical flask, and mixed. One ml each of 0.0125% 2,4-dinitrophenylhydrazine (2,4-DNPH) and deionised water were added to a 1 ml aliquot of homogenate/TCA/deionised water mixture and

vortexed for ca. 20 s. This mixture was heated in a water bath at 37°C for 10 min. Five ml of 0.6 N NaOH was then added and mixed. A UV/VIS spectrophotometer (Model PU8730; Unicam, UK) was used to measure absorbance at 420 nm (Schwimmer and Weston, 1961; Randle and Bussard, 1999). Two-way ANOVA for pyruvic acid concentration was performed using Minitab software. Treatment means were separated by the method of least significance difference (LSD) at the 5% level (Gomez and Gomez, 1984). A multiple linear regression analysis ($Y = \alpha + \beta_1 X_1 + \beta_2 X_2$; $N_{(X, Y)} = 6$) for pyruvic acid concentration (Y) with PC 1 (X_1) and PC 2 (X_2) as predictors (Gomez and Gomez, 1984) was also carried out using Minitab software.

RESULTS AND DISCUSSION

Water-deficit stress during growth influenced the E-nose sensor response (%dR/R; Table 1). Water-deficit at -1.19 MPa affected headspace volatiles resulting in increased sensor conductivity, i.e. smaller %dR/R values. Neither soil type nor soil type x irrigation interaction influenced %dR/R.

Headspace volatile fingerprints of the spring onion samples are represented by the 2D PCA plots in Fig. 1. The classification of clusters of data sets on the X-Y plane explained most of the total variance. The principal components, PC 1 (X co-ordinate) and PC 2 (Y co-ordinate) together accounted for more than 80% of the total variance in the data sets. Mahalanobis distance (D^2) statistics showed significant (D^2>3.0) separations among the sets of 2D PCA plots data clusters for irrigation regime, soil type and interaction of irrigation regime x soil type (Table 2). The relative classification of the data sets on the 2D PCA map for the clay (Fig. 1; upper panel) was different from that for the sandy loam (Fig. 1; lower panel). For the clay, the data set for spring onions subject to -0.01 MPa was located to the left of the data sets for -0.80 MPa (D^2=23.6) and -1.19 MPa (D^2=72.4). But for the sandy loam, the data set for plants subject to -0.80 MPa was located to the left of -0.01 MPa (D^2=49.7) and -1.19 MPa (D^2=59.6). These differences in cluster location can be attributed to the nature and/or intensity of headspace volatiles of the respective samples. The data sets for spring onions grown on the clay at -0.01 MPa versus plants grown on the sandy loam at -0.80 and for plants grown on the clay at -0.80 MPa versus plants grown on the sandy loam at -1.19 MPa were the closest. That is, their respective D^2 values were 3.3 and 3.0. The closeness of data set clusters of two or more samples in a PCA map suggest similarity in headspace volatile characteristics (Bartlett et al., 1997; Adechy et al., 2000). On average, increases in water-deficit stress level reduced separations between data set clusters of spring onions grown on clay and on sandy loam; i.e. -0.01 MPa, D^2=43.6; -0.80 MPa, D^2=30.3 and -1.19 MPa, D^2=6.4.

Soil type, irrigation level and their interactions influenced total pyruvic acid concentration (Table 3). Pyruvic acid concentrations in plants grown on the clay were generally slightly higher than those grown on the sandy loam. The difference was only large for plants grown on the clay at -0.01 MPa, hence the significant ($P<0.05$) interaction. Regular watering to -0.01 MPa markedly increased pyruvic acid concentration of plants grown on the clay by 33% as compared to plants subject to -0.80 MPa on the clay. In contrast, pyruvic acid concentration was reduced by 15% in plants subject to -0.01 MPa as compared to -0.80 MPa treatments on the sandy loam. This reduction in pyruvic acid concentration in spring onions subject to -0.01 MPa on the sandy loam cannot be readily explained. It is known that water-deficit stress limits root activity and, therefore, plant metabolism (Lambers et al., 1998), which may include biosynthesis of flavour precursors. The clay soil has higher moisture retention capacity due to its inherent physical properties (Rowell, 1996). Therefore, it could sustain root activity longer than the sandy loam. The greater growth (data not presented) and pyruvic acid concentration for plants grown on the clay tends to confirm greater root activity compared to the sandy loam.

Multiple linear regression (MLR) analysis suggested that linear combination of PC 1 and PC 2 did not significantly ($P>0.05$) explain variations in pyruvic acid concentration ($Y = 5.9 + 37.0$ PC 1 – 31.4 PC 2; r^2 = 58.1%; P = 0.40). That is, the MLR accounted for

only 58.1% of the total variation in pyruvic acid concentration of the spring onions affected by different water-stress levels and different soil types.

In conclusion, relative changes in sensor resistance (%dR/R) and spatial arrangements of treatment-specific data set clusters on the 2D PCA maps showed that the 32-conducting polymer sensor response and, therefore, spring onion volatiles are influenced by water-deficit stress treatment. Small but significant ($P<0.05$) changes in %dR/R were associated with low pyruvic acid concentration for severe water-deficit stressed spring onions, especially on the clay. This trend shows that a reduction in flavour intensity increases sensor conductivity by reducing %dR/R. Even otherwise, the 32-conducting polymer sensor E-nose successfully discriminated among spring onions grown under varying irrigation regimes for each soil type as shown in 2D PCA plots. Further work is required to identify specific chemicals in the headspace volatiles of the spring onions that interact with the sensors.

ACKNOWLEDGEMENT

The authors appreciate the technical assistance of Mr. Allen Hilton.

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Tables

Table 1. Relative changes in E-nose sensor resistance (%dR/R) for headspace volatiles of spring onion cv. White Lisbon grown on clay or sandy loam at three different soil water potentials (-0.01, -0.80 or -1.19 MPa).

SWP (MPa)	%dR/R		
	Clay	Sand	Row mean
-0.01	1.60	1.55	1.58a
-0.80	1.50	1.58	1.54a
-1.19	1.39	1.42	1.40b
Column mean	1.50a	1.52a	
$LSD_{(0.05)}$			
Soil type (A)		ns	
Irrigation (B)		0.04*	
A x B		ns	

*Significant at the 1% level; ns, not significant at 5% level; letters denote mean separation using $LSD_{0.05}$. Data were analysed by two-way ANOVA.

Table 2. Mahalanobis (D^2) distances on a 2D PCA plot (Fig. 1) between treatment data clusters of headspace volatiles of spring onion cv. White Lisbon grown on clay (C) and on sandy loam (S) at three different soil water potentials (-0.01, -0.80 and -1.19 MPa). Differences are significant at $D^2>2.0$.

	C, -0.01	C, -0.80	C, -1.19	SL, -0.01	SL, -0.80
C, -0.80	23.6	-	-	-	-
C, -1.19	72.4	6.1	-	-	-
SL, -0.01	43.6	9.8	18.3	-	-
SL, -0.80	3.3	30.3	62.9	49.7	-
SL, -1.19	56.7	3.0	6.4	12.9	59.6

Table 3. Pyruvic acid concentrations of spring onion cv. White Lisbon grown on clay or sandy loam at three different soil water potentials (-0.01, -0.80 or -1.19 MPa).

SWP (MPa)	Pyruvic acid concentration (µmole g^{-1} FW)		
	Clay	Sand	Row mean
-0.01	7.77a	4.94c	6.36
-0.80	5.86b	5.82b	5.84
-1.19	5.42bc	5.37bc	5.40
Column mean	6.35	5.38	
$LSD_{(0.05)}$			
Soil type (A)		0.35*	
Irrigation (B)		0.43*	
A x B		0.61**	

FW, fresh weight; *, **Significant at 5% and 1% levels, respectively; letters denote mean separation using $LSD_{0.05}$. Data were analysed by two-way ANOVA.

Figures

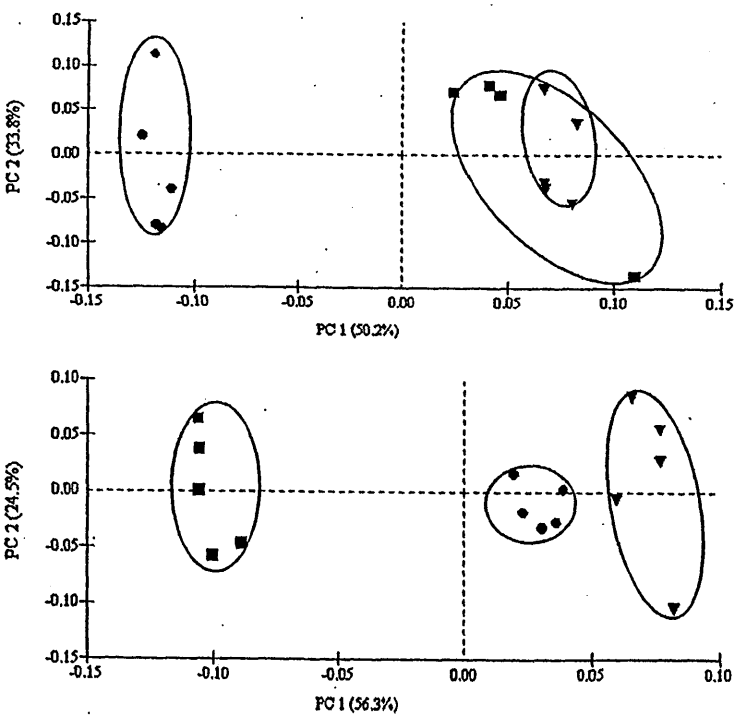

Fig. 1. Two-dimension (2D) Principal Component Analysis (PCA) plots for E-nose classification of spring onion cv. White Lisbon grown on clay (upper panel) and sandy loam (lower panel) at three different soil water potentials (SWPs) of -0.01 MPa (●), -0.80 MPa (■), and -1.19 MPa (▼).

Appendix III

Nitrogen, sulphur and soil type effects on growth and bulb scale leaf of onion (*Allium cepa* L. cv. Sprinters)

(In preparation)

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Abstract

Albeit fertiliser and soil type affect plant growth, their interaction effects on onion production and skin quality are not reported. Field and glasshouse pot experiments were instigated to determine N (field: 0, 120 kg ha^{-1}; glasshouse: 0, 108 kg ha^{-1}), S (field: 0, 20 kg ha^{-1}; glasshouse: 0, 4.35 kg ha^{-1}) and soil type (clay, sandy loam) effects on growth and quality of onion cv. Sprinters. N plus S fertilisation (+N+S) in the glasshouse reduced green leaf area duration by 8 days as compared with the average duration for the other fertiliser treatments. Leaf greenness SPAD value was increased by +N+S treatment from 8.0 to 51.1 (clay) and 55.4 (sandy loam) between 10 and 14 weeks after transplanting in the glasshouse. In the field, N fertilisation increased bulb fresh and dry weights by 3.57- and 2.10-fold, respectively, as compared with no N addition for all S levels. In the glasshouse, bulb fresh and dry weights for +N+S treatment rose by 5.45- and 3.13-fold on the clay and by 11.04- and 13.5-fold on the sandy loam, respectively, as compared with the average for the other treatments. Similarly, bulb diameter increased significantly ($P<0.05$) from 31.0 to 57.8 mm upon addition of N in the field. For the glasshouse clay and sandy loam, +N+S treatment gave the highest bulb diameters of 35.5 and 31.5 mm, respectively. Bulb firmness did not vary with treatment in the field but N addition significantly ($P<0.05$) increased numbers of fresh and dry scale leaves and dry scale leaf thickness. S-fertilised pot-grown plants had firmer bulbs, especially when combined with N on the sandy loam. Also, N, S and soil type and their interactions improved the number of fresh scale leaf only. Tissue N:water-soluble SO_4^{2-} ratios of 5 for the field and glasshouse sandy loams and 18 for the glasshouse clay corresponded with highest growth and bulb scale quality.

Keywords: *Allium cepa*, bulb yield, fertiliser, leaf area duration, scale leaf, skin thickness

Introduction

Onion is an important vegetable that is valued for the taste and aroma derived from organo-sulphur compounds during chewing (Block, 1992). Onions are comprised of considerable amounts of valuable nutrients (Rubatzky and Yamaguchi, 1997). For instance, a 140 g fresh bulb contains among others 14 g total carbohydrate; 2 g protein; 5 mg sodium; 4 mg calcium and 0.31 mg vitamin E (Caunce and Son, 2003). Genetic, management, soil and climatic variables affect the production and bulb quality of onions (Randle, 1997; Hamilton *et al.*, 1998; Sullivan *et al.*, 2001).

The major nutrients i.e. nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) are important in onion bulb production (Brewster, 1994). Nitrate-N and sulphate-S are soluble and easily leached from the rhizosphere or remobilised by soil microorganisms (Sullivan *et al.*, 2001) depending on the nature of the soil. The availability and abundance of soil nutrients are dependent on soil properties such as cation exchange capacity (CEC), which is greater in clay (i.e. between 4 and 60 $cmole_c\ kg^{-1}$) than in sandy soils (i.e. between 2 and 12 $cmole_c\ kg^{-1}$; Brady and Weil, 1996; Rowell, 1996). Onions are grown on wide range of soils from light to heavy soils and even in hydroponics (Ko *et al.*, 1993; Rubatzky and Yamaguchi, 1997; Randle, 2000). Despite a range of production soil types, onion plant growth, dry-matter content and bulb yield on sand versus clay soil can be similar (Patil *et al.*, 1995; Hamilton *et al.*, 1998). However, other workers reported higher growth of bulb onion (Talha *et al.*, 1978) and garlic (Hanson *et al.*, 2003) on clay than on sandy soil.

Application of excessive amount of N promotes leaf growth but depresses bulb swelling (Wilson, 1934). High N supply can thereby reduce fresh weight and also the firmness of onion bulbs (Randle, 2000). In another work, reductions in bulb growth due to high amount of N fertilisation were attributed to N toxicity (Sachdev *et al.*, 1991). Low N supply increases the bulb-to-leaf blade weight ratio (Bremer, 1936). Onion bulb yield is typically improved by increased S fertilisation, especially in combination with more N (Sachdev *et al.*, 1991; Singh *et al.*, 1996). This positive interaction is due to a mechanistic relationship between N and S (Reuveny *et al.*, 1980; Fox and Blair, 1986; Singh *et al.*, 1996). The activity of the key regulatory S assimilation enzyme, ATP *sulphurylase*, is enhanced by available nitrate. Conversely, activity of the key N assimilation enzyme, nitrate *reductase*, is enhanced by sulphate (Reuveny *et al.*, 1980).

Consumers generally judge onion quality on the basis of external appearance (Hole *et al.*, 2000). Good quality bulbs are expected to have no blemishes, such as discoloration, splits or

skin loss. Moreover, better tolerance of the rigours of postharvest handling and transportation is associated with an intact and thick skin of high tensile strength (Komochi, 1990; Hole *et al.*, 2000).

In spite of the above general understandings, there is a paucity of information on the interaction effects of N, S and/or soil type on onion bulb production and quality. The object of this work was to determine the responses of plant growth, bulb firmness and bulb skin quality of onion cv. Sprinters to N and S fertilisation in the field and to N, S and soil type in a glasshouse pot trial.

Materials and methods

Field and glasshouse investigations were carried out at Cranfield University, Silsoe, UK between May (spring) and December (winter). Seeds of bulb onion cv. Sprinters were obtained from Elsoms Seeds, Spalding for the study.

N and S field experiment

The experiment field sandy loam was classified as Yellowish Brown earth; Bearsted series (BC_u; Hodge *et al.*, 1984). The field was ploughed and harrowed on 25 May. The onion seeds were sown in drills at 20 cm between rows on 18 June. Seedlings were thinned by hand 2 weeks after emergence to a spacing of 10 cm within rows. Fertiliser treatments were 0 (-N) or 120 (+N, urea) kg N ha^{-1} and 0 (-S) or 20 (+S, magnesium sulphate) kg S ha^{-1}. Triple super phosphate at 125 kg P ha^{-1} and muriate of potash at 200 kg K ha^{-1} were applied to all the plots. The fertilisers were split, with half the rate applied at each of 3 and 5 weeks after sowing. Onion plants were watered weekly during summer when there was no rainfall using overhead sprinkler irrigation system. Bulbs were lifted early at 20% top-fall on 31 October due to wet weather conditions, which delayed planting and were prolonging bulb maturation associated with a rise in bulb rots. The experiment design was a randomised complete block factorial with four replications. The block size was 78.8 m^2. The blocks were split into two N levels (+N, -N; main plot factor) and two S levels (+S, -S; sub-plot factor) each of size 6.3 m^2. The gap in between blocks and plots were 1.0 and 0.5 m, respectively. Alleys 0.5 m wide occupied these gaps. Each plot comprised of three duplicate beds.

N, S and soil types glasshouse pot experiment

Onion seeds were pre-germinated in Petri dishes lined with moistened filter paper at 20°C from 2 to 7 June. The seedlings were then transplanted into 500 g clay or 700 g sandy loam in 12-cm diameter plastic pots. The clay soil was classified as Alluvial gley; Thames series

(T_s) and that of the sandy loam as Brown earths; Wick series (WQ_2; Hodge *et al.*, 1984). Both clay and sandy loam soils were collected from the Horticulture Research International (HRI) experimental station, Wellesbourne, UK. Each pot was thinned to only one onion plant. Fertiliser treatments were 0 (-N) or 108 kg N ha^{-1} (+N, urea; 120 mg N pot^{-1}) and 0 (-S) or 4.35 kg S ha^{-1} (+S, magnesium sulphate; 3.95 mg pot^{-1}). In addition, basal applications of 33 kg P ha^{-1} (24 mg P pot^{-1}) as metaphosphoric acid, 64 kg K ha^{-1} (72 mg K pot^{-1}) as muriate of potash and 21 kg ha^{-1} (24 mg Mg pot^{-1}) as magnesia were supplied to the plants. All fertilisers were in the form of nutrient solution. N and S nutrient solutions were applied in split applications 2 and 5 weeks after transplanting. The plants were irrigated with 196.5 ml (clay) and 104.4 ml (sandy loam) distilled water at -0.01 MPa soil water potential. Final harvest was on 12 December when 80% of the foliage leaves on plants grown in plots applied with both N and S fertiliser fell over. A split (factorial) randomised complete block design with three replications was used for the experiment. The main plot factor was soil type (clay, sandy loam), the subplot factor was two N levels (+N, -N) and the sub-sub plot factor was two S levels (+S, -S). Each replication was comprised of sixteen pots (plants) per treatment. Thus, the total number of plants for each treatment was 48 (i.e. 16 pots per replication by three blocks).

Data collection

Leaf greenness (chlorophyll content)

Leaf greenness (n = 6 or 8) was determined at 25% distance from the leaf base of the third or the fourth youngest leaf that had a diameter of ca. 5 mm. The leaf greenness SPAD value was recorded at 10 and 14 weeks after planting using a Minolta chlorophyll meter (model SPAD-501; Minolta Camera Co. Ltd., Japan).

Green leaf area duration and leaf elongation

For plants grown in the glasshouse, the third youngest true-leaf of ≤1 cm length was tagged and the total number of days to ≥50% dieback was recorded as green leaf area duration as per treatment (n = 12). Increases in the lengths of the tagged leaves (n = 12) were measured after every 4 days from 42 days after transplanting seedlings into pots in the glasshouse for 32 days using a 30-cm rule.

Foliage and bulb fresh and dry weights and harvest index

Total fresh weight of foliage including dead leaves (n = 24) was measured immediately after final harvest. Onion bulb fresh weight (n = 24) was recorded after sun and air curing the harvested bulbs between 20° and 36°C, 40 and 95% RH for 1 week on a bench in the

glasshouse. Foliage and bulbs were oven-dried at 70°C to constant weight for dry weight determination (n = 12). Harvest index (n = 12) was calculated as the ratio of bulb dry weight to total plant dry weight.

Bulb diameter and bulb firmness

Bulb diameter (n = 24) was measured from the broadest section ('equator') of the cured onion bulb using a digital electronic calliper (model RS 592095; Mitutoyo, Japan). Bulb firmness (n = 6 or 8) was measured at the equator of the bulb using a hand-held penetrometer (model FT 327; David Bishop Instruments, Sussex, UK) after bulb curing.

Numbers of fresh and dry scale leaves and thickness of dry scale leaf

The onion bulbs were cut transversely after the firmness test and the numbers of bulb fresh and dry scale leaves (n = 6 or 8) were counted with the aid of a hand lens of magnification x10. The total thickness of the dry scale leaves per bulb (n = 6 or 8) was measured using the digital electronic calliper.

Plant tissue N:water-soluble SO_4^{2-} ratio

Onion bulbs harvested from the field experiment and the whole plant (i.e. bulbs plus leaf tissues) harvested from the glasshouse experiment were oven-dried at 45°C to constant weight and ground. The ground samples were then analysed for N and water-soluble SO_4^{2-} contents (n = 2 or 3) using the Kjedahl and the Turbidimetric methods, respectively. The ratio N:water-soluble SO_4^{2-} ratio was then calculated.

Statistical analysis

Analyses of the general treatment structure for a randomised design ANOVA were performed using GenStat for Windows Version 4.21 (Rothamsted Experimental Station, UK) for each measured parameter. Least significance difference (LSD) at P = 0.05 values were used to separate treatment means (Gomez and Gomez, 1984).

Results

Soil analysis

The field sandy loam contained 1.2 g N and 1.5 g water-soluble SO_4^{2-} kg^{-1} between 0 and 40 cm depth before cultivation. For the glasshouse clay, N and water-soluble SO_4^{2-} concentrations were 68.7 g and 6.3 g kg^{-1} soil, respectively. And for the glasshouse sandy loam, N and water-soluble SO_4^{2-} concentrations were 31.6 g and 4.1 g kg^{-1} soil, respectively.

N x S field experiment

The N fertiliser treatment significantly ($P<0.05$) increased leaf greenness, plant growth, bulb yield and skin quality parameters of onion cv. Sprinters. Neither S treatment nor N x S interaction was significant ($P>0.05$). At 10 Weeks After Sowing (WAS) leaf greenness of plants that received 120 kg N ha^{-1} rose by 1.4-fold as compared with the 0 kg N ha^{-1} control treatment (Table 1). However, at 14 WAS the difference in leaf greenness was reduced. That is, at 14 WAS leaf greenness was 1.3-fold greater in the 120 kg N ha^{-1} treatment plot than in the 0 kg N ha^{-1} treatment plot as compared with 1.4-fold increase at 10 WAS.

Table 1. The effects of N and S fertilisation on leaf greenness SPAD value and fresh and dry weights of onion cv. Sprinters grown in the field.

N rate	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
(kg ha^{-1})	0	20	N mean	0	20	N mean
			Leaf greenness (SPAD value)			
	10 weeks after sowing			14 weeks after sowing		
0	38.0	40.2	39.1	43.0	46.5	44.8
120	57.9	62.3	60.1	57.4	59.3	58.4
S mean	48.0	51.0		50.2	52.9	
$LSD_{(0.05)}$	N^{**}, S^{ns} = 5.06; N x S^{ns} = 7.16			N^{**}, S^{ns} = 3.81; N x S^{ns} = 5.49		
			Fresh weight (g $plant^{-1}$)			
	Foliage			Bulb		
0	22.9	21.2	22.1	30.2	29.6	29.9
120	99.7	88.4	94.1	101.7	111.5	106.6
N mean	61.3	54.8		66.0	70.6	
$LSD_{(0.05)}$	N^{**}, S^{ns} = 10.35; N x S^{ns} = 14.63			N^{**}, S^{ns} = 22.01; N x S^{ns} = 31.13		
			Dry weight (g $plant^{-1}$)			
	Foliage			Bulb		
0	6.6	6.3	6.5	8.5	7.6	8.1
120	14.4	13.7	14.1	16.3	17.6	17.0
N mean	10.5	10.0		12.4	12.6	
$LSD_{(0.05)}$	N^{**}, S^{ns} = 0.95; N x S^{ns} = 1.34			N^{**}, S^{ns} = 2.95; N x S^{ns} = 4.17		

**Significant at $P<0.01$; ^{ns}not significant at $P>0.05$; number of observations (n) used to calculate $LSD_{(0.05)}$ were 8 for N, S and 4 for N x S.

Application of N plus S (+N+S) or only N (+N-S) increased foliage and bulb fresh and dry weights significantly ($P<0.01$; Table 1). For both foliage and bulbs that were harvested from the 120 kg N ha^{-1} treatment plot, increases in fresh and dry weights were >1.7- and >1.5-fold, respectively, as compared with the 0 kg N ha^{-1} control treatment. The effects of no fertiliser

addition treatment (-N-S) versus application of S alone (-N+S) on fresh and dry weights of foliage and bulb did not differ. The Harvest Indices, HIs calculated from the ratio of bulb to total plant dry weights were all similar (Table 2).

Table 2. Effects of N and S fertilisation on harvest index, bulb diameter, and bulb firmness of onion cv. Sprinters grown in the field.

N rate	S rate (kg ha^{-1})		
(kg ha^{-1})	0	20	N mean
	Harvest index (HI)		
0	0.56	0.55	0.56
120	0.53	0.56	0.55
S mean	0.55	0.56	
$LSD_{(0.05)}$	N^{ns}, S^{ns} = 0.054; N x S^{ns} = 0.077		
	Bulb diameter (mm)		
0	32.6	29.4	31.0
120	56.0	59.6	57.8
S mean	44.3	44.5	
$LSD_{(0.05)}$	N^{**}, S^{ns} = 4.07; N x S^{ns} = 5.76		
	Bulb firmness (kg)		
0	4.6	5.1	4.9
120	4.7	4.8	4.8
S mean	4.7	5.0	
$LSD_{(0.05)}$	N^{ns}, S^{ns} = 1.29; N x S^{ns} = 1.83		

** Significant at P<0.01; ^{ns}not significant at P>0.05; number of observations (*n*) used to calculate $LSD_{(0.05)}$ were 8 for N, S and 4 for N x S.

There was 1.86-fold increase in bulb diameter for N-fertilised plants as compared with no added N control treatment, irrespective of S level (Table 2). There was no significant (P>0.05) N x S interaction. N, S or N x S did not influence bulb firmness (Table 2).

The N fertilisation significantly (P<0.05) increased the numbers of fresh and dry scale leaves (Table 3). Although S fertilisation did not significantly (P>0.05) affect the number of bulb fresh scale leaf, N x S interaction was significant (P<0.05). Thus, the number of fresh scale leaves was 1.14-fold greater for the +N+S treatment than for the +N-S treatment. Treatments -N-S and -N+S had similar effects on number of fresh scale leaf and was 35% less than that for the +N+S treatment. The +N+S treatment also increased the number of outer dry scale leaves significantly (P<0.05) by one, but the number remained the same for all the other three treatments (Table 3). The outer bulb dry scale leaf thickness was significantly (P<0.05)

increased when 120 kg N ha^{-1} was applied (Table 3). Both S fertilisation and N x S interaction were not significant (P>0.05) for either the number or thickness of dry scale leaves.

Table 3. Effects of N and S fertilisation on number of fresh and dry scale leaves, thickness of dry scale leaves and bulb tissue N:water-soluble SO_4^{2-} ratio of onion cv. Sprinters grown in the field.

N rate	S rate (kg ha^{-1})		
(kg ha^{-1})	0	20	N mean
	Number of fresh scale leaf		
0	9	9	9
120	12	14	13
S mean	11	12	
$LSD_{(0.05)}$	N^{**}, S^{ns} = 1.2; N x S^{*} = 1.68		
	Number of dry scale leaf		
0	2	2	2
120	2	3	3
S mean	2	3	
$LSD_{(0.05)}$	N^{*}, S^{ns} = 0.3; N x S^{ns} = 0.41		
	Thickness of dry scale leaf (mm)		
0	0.298	0.367	0.333
120	0.616	0.834	0.725
S mean	0.457	0.601	
$LSD_{(0.05)}$	N^{*}, S^{ns} = 0.174; N x S^{ns} = 0.250		
	Bulb tissue N:water-soluble SO_4^{2-} ratio		
0	8:1	8:1	
120	5:1	5:1	

*,** Significant at P<0.05 and P<0.01, respectively; ^{ns}not significant at P>0.05; number of observations (*n*) used to calculate $LSD_{(0.05)}$ were 8 for N, S and 4 for N x S.

The ratio of bulb tissue N:water-soluble SO_4^{2-} concentration varied for the N fertiliser treatment (Table 3). The bulb tissue N:water-soluble SO_4^{2-}ratio reduced upon application of N fertiliser but remained the same for the different S fertiliser treatment.

N x S x soil type glasshouse experiment

The greatest leaf greenness SPAD value was recorded at 10 Weeks After Transplanting (WAT) for both clay and sandy loam supplied with +N+S fertiliser (Table 4). At 10 WAT, the SPAD value was the same and the least due to +N-S treatment on both soils. At 14 WAT, the relatively low SPAD value of 8.0 for the +N-S treatment significantly (P<0.01) increased

by factors of 6.4 on the clay and 6.9 on the sandy loam. There was an overall increase in leaf greenness SPAD values between 10 and 14 WAT, especially for -N-S, -N+S and +N-S treatments on the sandy loam. All interactions were not significant (P>0.05).

Table 4. The effects of N, S and soil type on leaf greenness, green leaf area duration and foliage fresh weight on bulb onion cv. Sprinters grown in a glasshouse.

N rate	Clay			Sand		
(kg ha^{-1})	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
	0	4.35	N mean	0	4.35	N mean
Leaf greenness (SPAD value) at 10 weeks after transplanting						
0	43.3	44.7	44.0	35.0	34.0	34.5
108	8.0	61.0	34.5	8.0	59.0	33.5
S mean	25.6	52.9	39.3^{C}	21.5	46.5	34.0^{S}
$LSD_{(0.05)}$:	N^{*}, S^{*}, $Soil^{*}$ = 4.07; N x S^{**}, N x $soil^{*}$, S x $soil^{ns}$ = 5.76; N x S x $soil^{ns}$ = 8.14					
Leaf greenness (SPAD value) at 14 weeks after transplanting						
0	47.7	42.9	45.3	55.4	39.9	47.7
108	51.1	51.5	51.3	55.4	56.2	55.8
S mean	49.4	47.2	48.3^{C}	55.4	48.1	51.8^{S}
$LSD_{(0.05)}$:	N^{**}, S^{ns}, $Soil^{ns}$ = 3.51; N x S^{ns}, N x $soil^{ns}$, S x $soil^{ns}$ = 4.97; N x S x $soil^{ns}$ = 7.02					
Green leaf area duration (GLAD; day)						
0	75	74	75	81	81	81
108	78	64	71	77	68	73
S mean	77	69	73^{C}	79	75	77^{S}
$LSD_{(0.05)}$:	N^{**}, S^{**}, $Soil^{**}$ = 2.9; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 4.1; N x S x $soil^{ns}$ = 5.8.					
Foliage fresh weight (g plant^{-1})						
0	4.1	4.7	4.4	1.5	1.4	1.5
108	3.2	10.1	6.7	1.3	7.7	4.5
S mean	3.7	7.4	5.6^{C}	1.4	4.6	3.0^{S}
$LSD_{(0.05)}$:	N^{**}, S^{**}, $Soil^{**}$ = 1.36; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 1.92; N x S x $soil^{ns}$ = 2.72					

$^{C, S}$Soil mean for clay and sandy loam, respectively; *, **Significant at P<0.05 and P<0.01, respectively; ^{ns}not significant at P>0.05; number of observations (*n*) used to calculate $LSD_{(0.05)}$ were 12 for N, S , soil, 6 for N x S, N x soil, S x soil and 3 for N x S x soil.

The Green Leaf Area Duration (GLAD) of onion cv. Sprinters was increased significantly (P<0.01) with -N-S, -N+S and +N-S treatments as compared to +N+S treatment, especially on the sandy loam (Table 4). Only the N x S interaction was significant (P<0.01) as compared to all the other treatment interactions. The GLAD for -N-S and -N+S treatments was the same for each soil type.

Plant growth measured in terms of leaf elongation was significantly ($P<0.01$) greater in onions that were grown on +N+S treatment plots as compared with treatments +N-S, -N+S and -N-S on both clay and sandy loam (Fig. 1). After 52 days of transplanting, the increase in

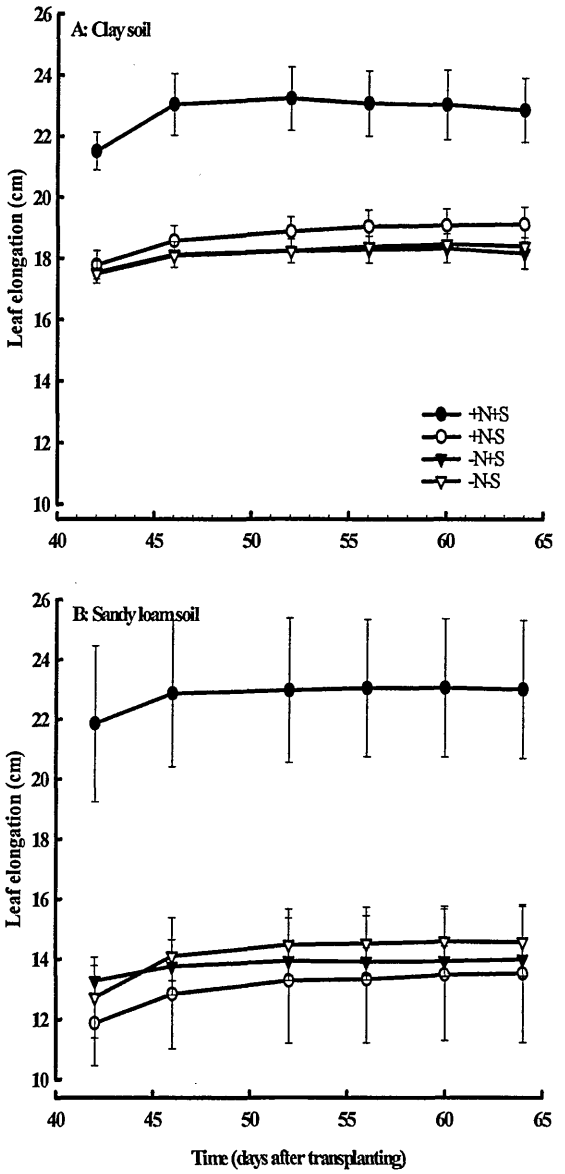

Fig. 1. The effects of N and S fertilisation on leaf elongation of onion cv. Sprinters grown on clay (A) and on sandy loam (B) in pots in the glasshouse.

leaf elongation due to +N+S treatment on the clay plot begun to reduce as compared with the +N+S sandy loam plot, which maintained a relatively steadily rate of increase until after 64 days. There were no significant ($P>0.05$) variations on the effects of treatments +N-S, -N+S

and -N-S on leaf elongation for either the clay or sandy loam. However, the average leaf length due to these three fertiliser treatments was greater on the clay than on the sandy loam.

Foliage and bulbs fresh and dry weights were increased significantly ($P<0.01$) by N and S fertilisation (Table 5). However, N alone treatment consistently reduced these growth and yield parameters. +N+S increased these growth and yield parameters, especially on the clay

Table 5. The effects of N, S and soil type on foliage dry weight, bulb fresh and dry weights and harvest index of bulb onion cv. Sprinters grown in a glasshouse.

N rate	Clay			Sand		
(kg ha^{-1})	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
	0	4.35	N mean	0	4.35	N mean
			Foliage dry weight (g plant^{-1})			
0	0.7	0.8	0.8	0.2	0.2	0.2
108	0.6	2.0	1.3	0.2	1.5	0.9
S mean	0.7	1.4	1.1^{C}	0.2	0.9	0.6^{S}
$LSD_{(0.05)}$:	N^{**}, S^{**}, $Soil^{**}$ = 0.25; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 0.35; N x S x $soil^{ns}$ = 0.50					
			Bulb fresh weight (g plant^{-1})			
0	5.5	5.3	5.4	2.0	1.9	5.4
108	3.4	25.8	14.6	1.4	19.5	10.5
S mean	4.5	15.6	10.0^{C}	2.7	10.7	6.7^{S}
$LSD_{(0.05)}$:	N^{**}, S^{ns}, $Soil^{**}$ = 2.61; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 3.69; N x S x $soil^{ns}$ = 5.22					
			Bulb dry weight (g plant^{-1})			
0	1.0	0.8	0.9	0.2	0.3	0.3
108	0.5	2.4	1.5	0.1	2.7	1.4
S mean	0.8	1.7	1.2^{C}	0.2	1.5	0.9^{S}
$LSD_{(0.05)}$:	N^{**}, S^{**}, $Soil^{*}$ = 0.46; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 0.65; N x S x $soil^{ns}$ = 0.93					
			Harvest index (HI)			
0	0.57	0.50	0.54	0.56	0.56	0.56
108	0.50	0.61	0.56	0.38	0.65	0.52
S mean	0.54	0.56	0.55^{C}	0.47	0.61	0.54^{S}
$LSD_{(0.05)}$:	N^{ns}, S^{ns}, $Soil^{ns}$ = 0.98; N x S^{*}, N x $soil^{ns}$, S x $soil^{ns}$ = 0.139; N x S x $soil^{ns}$ = 0.196					

$^{C, S}$Soil mean for clay and sandy loam, respectively; *, **Significant at $P<0.05$ and $P>0.01$; ^{ns}not significant at $P>0.05$; number of observations (n) used to calculate $LSD_{(0.05)}$ were 12 for N, S , soil, 6 for N x S, N x soil, S x soil and 3 for N x S x soil.

as compared with the sandy loam and plots treated with -N-S, -N+S and +N-S. The fresh and dry weights of the foliage and bulbs were similar for -N-S, -N+S and +N-S treatments. HI

did not differ significantly ($P>0.05$) among treatments (Table 5). Nonetheless, the N x S interaction was significant ($P<0.05$) for HI as compared with all the other interactions.

Bulb diameter was increased significantly ($P<0.01$) following N and S fertilisation, interactions between N and S and the clay soil as compared with the other interactions and the sandy loam (Table 5). Bulb diameters for onions grown on the clay were increased by a factor of 1.93 upon addition of +N+S fertiliser while those on the sandy loam were increased by a factor of 2.48 as compared with the average for +N-S, -N+S and -N-S treatments on the respective soils. Overall, bulb diameter of onions harvested from the clay was 1.3-fold greater than those harvested from the sandy loam. The N fertilisation did not affect bulb firmness. Bulb firmness was, however, increased significantly ($P<0.01$) by S fertilisation on both soils (Table 6). Bulb firmness was generally greater on the clay as compared with the sandy loam. The greatest increase in bulb firmness was found upon application of +N+S to plants growing on the sandy loam. A lesser but still significant ($P<0.05$) treatment effect was also recorded on the clay.

The greatest number of fresh scale leaf was recorded for the +N+S treatment and the smallest number +N-S treatment plots (Table 6). There were no significant ($P>0.05$) differences in the numbers of fresh bulb scale leaf for the -N-S versus the -N+S treatment on both clay and sandy loam. The number (i.e. 4 for all the fertiliser treatments; data not shown) and thickness (Table 6) of dry scale leaves did not vary significantly ($P>0.05$) in response to N, S or soil type treatment.

N fertilisation increased the ratio of total plant (foliage plus bulb) tissue N:water-soluble SO_4^{2-} concentration irrespective of S treatment across soil type (Table 6). In contrast, S fertilisation reduced tissue N:water-soluble SO_4^{2-} ratio irrespective of N treatment except for no added N control treatment on the clay soil. Thus, for no N addition treatment on the clay soil, the N:water-soluble SO_4^{2-} ratio increased with S fertilisation.

Table 6. The effects N, S and soil type on bulb diameter, bulb firmness, thickness of dry scale leaf, number of fresh scale leaf and total plant tissue N:water-soluble SO_4^{2-} ratio of onion cv. Sprinters grown in a glasshouse.

N rate	Clay			Sand		
(kg ha^{-1})	S rate (kg ha^{-1})			S rate (kg ha^{-1})		
	0	4.35	N mean	0	4.35	N mean
			Bulb diameter (mm)			
0	19.8	18.0	18.9	12.2	13.5	12.9
108	17.4	35.5	26.5	12.3	31.5	21.9
S mean	18.6	26.8	22.7^{C}	12.3	22.5	17.4^{S}
$LSD_{(0.05)}$:	N^{**}, S^{**}, $Soil^{**}$ = 2.73; N x S^{**}, N x $soil^{ns}$, S x $soil^{ns}$ = 3.86; N x S x $soil^{ns}$ = 5.46					
			Bulb firmness (kg)			
0	1.03	1.32	1.18	0.93	0.91	0.92
108	1.05	1.36	1.20	0.76	1.46	1.11
S mean	1.04	1.34	1.19^{C}	0.85	1.19	1.02^{S}
$LSD_{(0.05)}$:	N^{ns}, S^{**}, $Soil^{*}$ = 0.14; N x S^{*}, N x $soil^{ns}$, S x $soil^{ns}$ = 0.20; N x S x $soil^{*}$ = 0.29					
			Thickness of dry scale leaf (mm)			
0	0.310	0.270	0.290	0.367	0.312	0.340
108	0.292	0.272	0.282	0.213	0.295	0.254
S mean	0.301	0.271	0.286^{C}	0.290	0.304	0.297^{S}
$LSD_{(0.05)}$:	N^{ns}, S^{ns}, $Soil^{ns}$ = 0.074; N x S^{ns}, N x $soil^{ns}$, S x $soil^{ns}$ = 0.105; N x S x $soil^{ns}$ = 0.148					
			Number of fresh scale leaf			
0	10	10	10	6	7	7
108	7	12	10	5	14	10
S mean	9	11	10^{C}	6	11	9^{S}
$LSD_{(0.05)}$:	N^{*}, S^{**}, $Soil^{**}$ = 0.92; N x S^{**}, N x $soil^{**}$, S x $soil^{*}$ = 1.30 N x S x $soil^{ns}$ = 1.84					
			Total plant tissue N:water-soluble SO_4^{2-} ratio			
0	5:1	8:1		10:1	4:1	
120	44:1	18:1		12:1	5:1	

$^{C, S}$Soil mean for clay and sandy loam, respectively; *, **Significant at P<0.05 and P<0.01, respectively; ^{ns}not significant at P>0.05; number of observations (*n*) used to calculate $LSD_{(0.05)}$ were 12 for N, S , soil, 6 for N x S, N x soil, S x soil and 3 for N x S x soil.

Discussion

In contrast with bulb onion cv. Sprinters grown in the glasshouse pot experiment (Tables 4 and 5), S fertilisation in the field experiment did not influence plant growth and bulb yield (Tables 1 and 2). S probably became deficient in the confined volume of the pots. As a consequence, plants responded to variations in N versus S in the glasshouse.

The lower leaf greenness SPAD value of field-grown onions that did not receive N fertiliser, irrespective of S level, can be ascribed to less chlorophyll (Street and Kidder, 1997). The small SPAD value of 8.0 at 10 WAT for plants that received only N on clay and sandy loam soils in the glasshouse seemed to be transient (Table 4). At 14 WAT, leaf greenness for these plants rose by >6-fold, and was not significantly ($P>0.05$) different from the combined N and S treatment on both soil types. The N fertilisation probably lowered soil pH (Sullivan *et al.*, 2001) that subsequently improved soil SO_4^{2-} availability for roots uptake (Fox and Blair, 1986). This may explain the improvement in leaf greenness of N-fertilised plants at 14 WAT. Initially low leaf greenness values for N and/or S deprived onion plants coincided with stage of bulb initiation (Tables 1 and 4). Bulb formation and swelling usually occur between 70 and 90 days after planting (Sullivan *et al.*, 2001). N and/or S limitation reduced plant growth and leaf turnover measured in terms of leaf elongation (Fig. 1) and green leaf area duration (Table 4). Assimilate production was reduced in inadequately fertilised plants as previously reported (Buwalda and Freeman, 1987; Brewster and Butler, 1989; Hawkesford, 2000). As a result of low assimilate accumulation during bulbing, the significant ($P<0.01$) reductions in foliage and bulb fresh weights (Tables 1 and 5) and bulb diameter (Tables 2 and 6) for N and/or S deprived plants as compared with the N plus S treatment may be expected.

The number of bulb fresh scale (storage) leaf can determine bulb size (Rubatzky and Yamaguchi, 1997). Deficiencies in N, S or both reduced the number of fresh scale leaf (Tables 3 and 6) as well as bulb diameter. The proportion of total dry-matter harvested as yield (i.e. the Harvest Index, HI), did not vary with N or S fertilisation or soil type (Tables 2 and 5). The overall HI was 0.55, which suggested that 55% of the total dry-matter content of the plant was partitioned into the bulb at final harvest.

N and S fertilisation in the field did not affect onion bulb firmness (Table 3). The absence of significant ($P<0.05$) N effect on bulb firmness in both the field and the glasshouse pot experiments can perhaps be attributed to adequate inherent soil N for bulb firmness. Randle (2000) found that above certain N threshold, bulb firmness is reduced. In contrast to the field experiment, S-fertilised bulbs grown in pots in the glasshouse were firmer than S-deprived bulbs. The properties of the clay soil enhanced bulb firmness by a factor of 1.15 as compared with the sandy loam.

The number or thickness of dry scale leaves was not markedly influenced by fertiliser treatments or soil type. Both quality parameters were slightly but significantly ($P<0.05$) increased with N fertiliser addition in the field sandy loam (Table 3). These quality

parameters were, however, not altered in the glasshouse pot experiment. This suggests that both number and thickness of dry scale leaves may largely be determined by genotype.

The tissue N:water-soluble SO_4^{2-} ratio indicated that a ratio of 5 for plants grown on sandy loam soil either in the field or glasshouse corresponded to higher growth, bulb yield and leaf scale quality (Tables 3 and 6). For the clay soils, a ratio of 18 enhanced these growth and yield parameters (Table 6). Thus, ratios more or less than 5 for the sandy loam and 18 for the clay suggest excessive or inadequate supply of N or S required for higher growth and assimilate production and improved scale leaf formation.

In conclusion, the significance ($P<0.05$) of N, S and soil type and their interactions on plant growth, yield and bulb skin quality of onion cv. Sprinters were demonstrated. Overall, N fertilisation increased plant growth and bulb yield. In contrast with the field experiment, N alone in the glasshouse depressed bulb diameter, and foliage and bulb fresh and dry weights. Bulb skin thickness was increased significantly $P<0.05$) by N fertilisation in the field experiment. S fertilisation had little or no significant ($P>0.05$) effect on growth and skin quality parameters in the field. Nonetheless, S fertilisation in the glasshouse pot experiment significantly ($P<0.01$) increased plant growth, bulb diameter, bulb firmness and the number of fresh scale leaf. These suggest the possibility of improving onion storage ability and tolerance to postharvest handling by S fertilisation. Although onions can be grown on a wide range of soil, it was found that constraints such as N and/or S limitation and roots growth restrictions reduce plant growth and bulb quality more on sandy loam than on clay in the glasshouse pot experiment. Onion tissue N:water-soluble SO_4^{2-} ratio required for improving growth, yield and bulb skin quality was found to be 18 for plants grown on the clay and 5 for those on the sandy loam in both field and glasshouse.

Acknowledgement

The authors are grateful for technical assistance from Mr. Allen Hilton.

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Appendix IV: The Glutathione Pathway

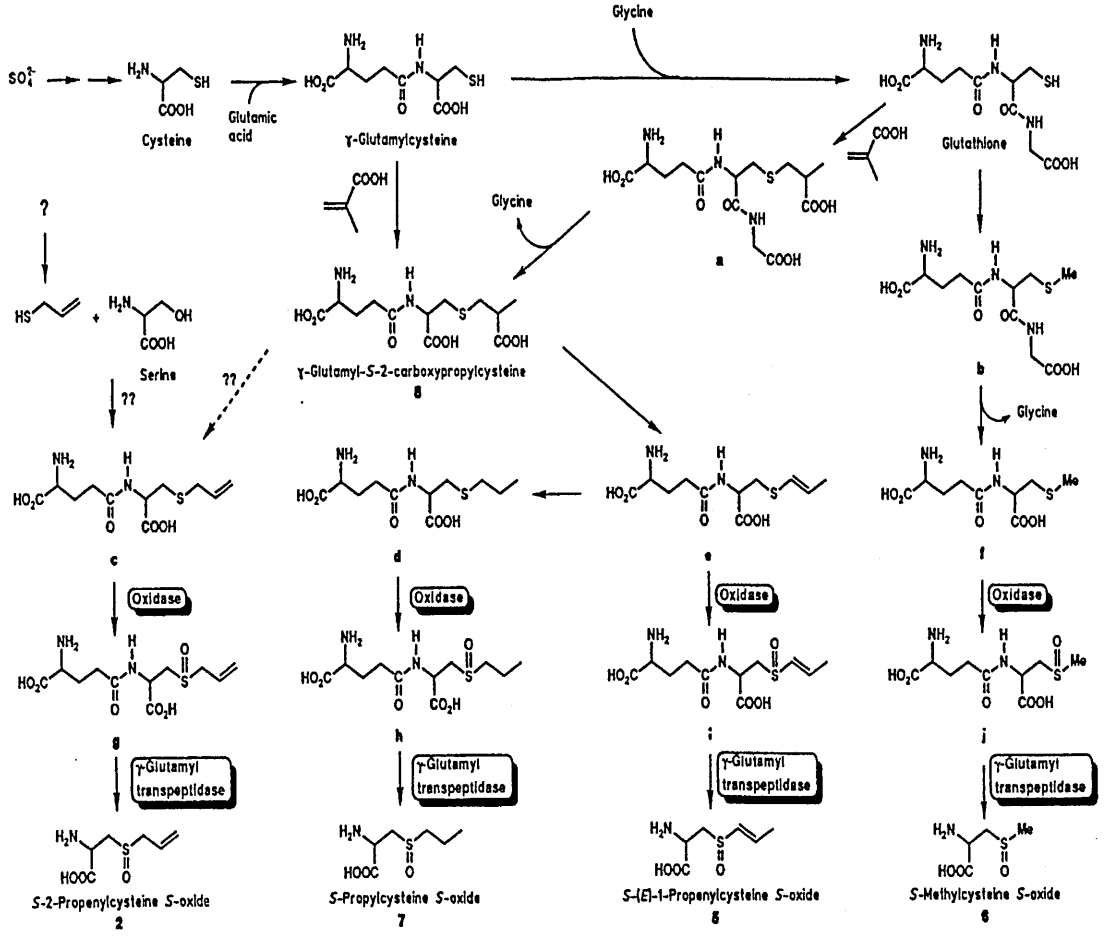

Figure 1. The glutathione pathway showing the major steps involved in the biosynthesis of flavour precursor compounds of *Allium* species. The peptides a = S-2-carboxypropylglutathione, b = S-methylglutathione, c = γ-glutamyl-S-2-propenylcysteine, d = γ-glutamyl-S-2-propylcysteine, e = γ-glutamyl-S-1-propenylcysteine, f = γ-glutamyl-S-methylcysteine, g = γ-glutamyl-S-2-propenylcysteine S-oxide, h = γ-glutamyl-S-2-propylcysteine S-oxide, i = γ-glutamyl-S-1-propenylcysteine S-oxide and j = γ-glutamyl-S-methylcysteine S-oxide (after Block, 1992).

Appendix V: A 2D Principal Component Analysis Plot of the Effect of Trichloroacetic Acid Treatment on Onion Headspace Volatiles Evaluated Using an E-Nose

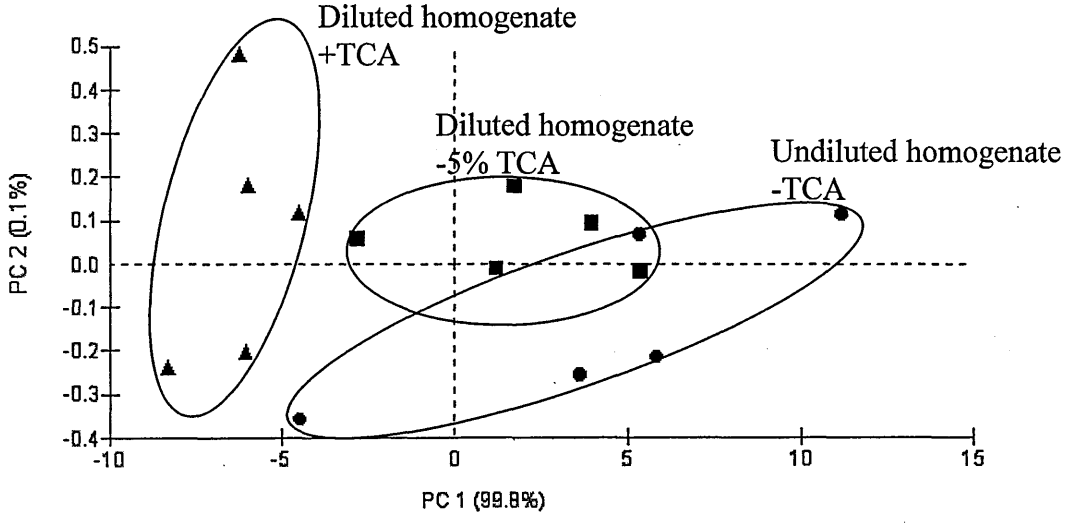

Figure 2. A 2D Principal Component Analysis plot of a 32 conducting polymer sensor type E-nose data sets for onion headspace volatile clusters: (a) undiluted onion homogenate (b) diluted homogenate with deionised water (1:1 v/v) alone (c) diluted homogenate with deionised water (1:1 v/v) plus 5% TCA. The three E-nose data set clusters were different. Addition of 5% TCA in treatment (a) produced a cluster that was significantly (D^2>3.0) different from clusters for treatments (b) and (c), which partly overlapped. The location of cluster (a) is in conformity with treatments that resulted in stronger *Allium* flavour strengths reported in this thesis (*see* Chapters 3 to 5 for details).